VOLUME LXXXVI NUMBER 1

THE

BOTANICAL GAZETTE

September 1928

ASEXUAL REPRODUCTION IN COLEOCHAETE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 382

OPHELIA C. WESLEY

(WITH PLATES I, II AND FORTY-ONE FIGURES)

Introduction

Coleochaete occupies a position of peculiar interest and importance, because of the various stages in the transition from the branched filamentous form to that of the many-celled disk, because of the covering developed from the surrounding cells around the fertilized egg, and because of the cellular body produced by divisions of the fertilized egg. This many-celled body, which is the nearest approach in the algae to the simplest sporophyte found in the liverworts, has attracted much attention in the past. Since no extended and critical work has been done following the classic work of PRINGSHEIM (13) in 1854, JOST (5) in 1895, and OLTMANN (11) in 1898, it seemed worthwhile to cover the genus, as much as possible, both morphologically and cytologically, applying more modern methods of technique and using the latest microscopical equipment.

While it is planned to cover the entire life history of every species of the genus in this investigation, the present paper takes up only that part of the life history from the formation of the zoospore produced by the large thallus to the formation of another mature thallus. Only four species will be considered at this time, *Coleochaete irregularis*, *C. soluta*, *C. orbicularis*, and *C. scutata*. In a paper to follow soon the sexual stages of these four species will be discussed. The material on hand is not sufficient for the different stages of the

entire life histories of *C. pulvinata* and *C. nitellarum*, but it is hoped that such material may soon be available, in order that the study of these two may be completed. So far it has been impossible to obtain material for the study of *C. divergens* and the two Australian species *C. baileyii* and *C. conchata*, so that these too must await further collection and work.

Much of the ground covered by this investigation has been covered before, but in order that the life history might be as complete as possible, it was deemed advisable to reinvestigate the entire life history, hoping to detect any errors that might have crept into the accounts, and to discover such additional facts as had escaped observation. In order to bring all the information into as full and connected a form as possible, it was thought best to give an account of the work done at this time without reference to any previous work. All such work will be given credit in the discussion, and all new work will be listed in the summary, thus making it possible to obtain a full understanding of the genus, even by one entirely unfamiliar with the subject, while one who is familiar with it may obtain the new points without reading the entire paper.

Materials and methods

Material for this work was collected in a lagoon in Washington Park in Chicago, and in a river at Miller, Indiana. The collections in Washington Park were made weekly from July 6, 1926, to November 30, 1927, after which they were made monthly until April, 1927. The collections at Miller were made at irregular intervals from June 18 until October 30, 1927. The material collected in Washington Park was found growing on *Sagittaria*, while that at Miller grew on *Typha*.

SIZE, CONDITION, AND DISTRIBUTION.—The thalli range in size from one cell to 5 mm. in diameter in *C. scutata*, one cell to 0.065 mm. in *C. soluta*, one cell to 0.06 mm. in *C. orbicularis*, while in *C. irregularis* it spreads in an irregular mass hard to estimate, for often quite long filaments run out from it. The cells vary in size in *C. scutata* from 13.2–25 μ in width, 15.4–30 μ in length; *C. soluta* from 7.3–11.7 μ in width, 8–26.4 μ in length; *C. orbicularis* from 4.4–10 μ in width, 8–13.2 μ in length; *C. irregularis* from 6.6–15.4 μ in width,

8.8–16.9 μ in length. *C. orbicularis* can always be distinguished from *C. soluta* by its more regular outline, the dorsal placing of the hairs, and its regular method of cell division, as contrasted with *C. soluta*, whose outline is very irregular, whose hairs arise from the side of the cell, and whose cell wall pushes in from the outside, following the line of division of the chloroplast. *C. scutata* and *C. irregularis* could not be confused with any of the other species.

From the time of the beginning of the collections until November 30, 1926, the thalli increased in size and number, while from that time until the return of warm weather no new thalli were formed, nor was there any considerable growth. From November 30, 1926, until March, 1927, thalli were taken from under and from the ice, and these proved to be in perfect vegetative condition, for the second morning after being brought in they were producing zoospores in abundance; in fact, thalli that had been frozen in the ice for months, when brought into the warm room in February began the production of great numbers of zoospores.

The material was very abundant from just below the water's edge to about 6 inches below the surface. All four species were often found in the low power field. Sometimes the thalli extended farther down, but nearly always in much less abundance. Light seemed to be the determining factor, because a petiole or leaf, as the case might be, had few if any thalli on the shady side, whereas when a plant to which *Coleochaete* was attached stood in the open its petioles would often be covered on both sides.

PREPARATION OF MATERIAL.—*Sagittaria* petioles were split open and placed with the *Coleochaete* side next to a piece of glass, and were scraped with a scalpel, removing nearly all of the cells of the petiole except the epidermal ones. By this means the substratum was made thin enough to stain and still not materially obstruct the view. This method of treatment proved impossible for *Typha*, and all habit material was removed from that with a safety razor blade. Since the thalli of *C. scutata* on *Typha* were especially large this was possible. The thalli became very brittle in the higher grades of alcohol and xylol, hence they were carried through these in glass tubes, one of whose ends was covered with a piece of fine bolting silk. In this way they could be handled with little or no injury.

Material separated from the substratum in this way, as well as pieces of the epidermis of *Sagittaria*, were placed in tap water in large shallow glass dishes whose flat bottoms had been covered with microscope slides. These were then placed in the sun, and as the zoospores were formed many of them settled on the slides and attached themselves so firmly that they were killed, stained, washed, run up, and mounted just like a slide of sections. As the hot summer sun killed not only the zoospores but the mature plants as well, the early fall proved best for preparation of such mounts.

KILLING AND STAINING.—Stock chromo-acetic solution, weak chromo-acetic, formalin-acetic-alcohol, formalin-acetic-water, and distilled formalin-acetic-water were used to kill in. Osmic was added to the stock chromo-acetic solution when figures were desired. That killed in stock chromo-acetic proved entirely satisfactory, hence was most used. Fresh material when compared with that killed in it showed the same structure and arrangement of parts of the protoplasm. At all times when possible fresh and fixed material were compared, in order to avoid, as nearly as possible, mistakes due to reactions to the killing agent. Although other stains were used, Haidenhain's iron-alum haematoxylin gave the best details of structure. Power's carmine stain for sporelings gave remarkably clear transparent and well differentiated mounts. Orange G was frequently used as a counter stain.

MOUNTING AND IMBEDDING.—The material to be mounted whole was run through the different grades of alcohol and xylol, just as paraffin sections are, and mounted in balsam. Since the pieces of *Sagittaria* and the separated thalli became very brittle and warped, an extra supply of balsam was necessary; therefore some was placed on both sides and a clip attached to the mount to flatten out the piece and get rid of the surplus balsam.

The usual routine method was followed in running up the material for imbedding. Narrow strips of cheese cloth were used to suspend the paraffin in the shell. By this means a piece of paraffin large enough for complete infiltration could be suspended at any depth, thus avoiding crushing the material. After from 4 days to 4 weeks on top of the oven, the material was poured into copper trays $0.75 \times 1 \times 6$ inches and covered with parawax. This was

changed four times in the 45–60 minutes they were kept on a specially constructed transite box whose temperature ranged from 52° at one end to 70° C. at the other. Thus the temperature was kept at from 52–53° C. during the entire time. The final imbedding was done in paraffin that had been heated to the smoking point and kept there for 6 or 7 hours. This gave a very homogeneous paraffin that could be cut at from 3–10 μ.

Zoospore production

FORMATION OF OPENING.—The normal position of the platelike chloroplast is flat against the dorsal wall, covering nearly all of the exposed surface (fig. 2 *X*). Wherever an opening is being formed, the chloroplast is pressed against the wall in the spot just below the place where the opening will appear; the remainder of the plastid stands away from the wall. The plastid at the point of contact shows a much denser, more darkly staining region (fig. 3). The first indication of any opening in the wall is a lighter staining area just above the darker region on the chloroplast. All around this region the wall gives the normal reaction to the stain (fig. 3 *b*). A later stage shows an opening where the light staining area has been. This opening is closed only by a new very thin wall which the cytoplasm has laid down on the outside (fig. 5 *w*). This wall is attached to the lateral walls of the cell. The opening when complete has sometimes a smooth outline and sometimes a ragged one (figs. 4, 5). The opening seems to be due to some chemical reaction, probably enzymatic. Although this was not definitely proved, all indications point to a chemical reaction.

ESCAPE OF ZOOSPORE.—Soon after the opening is formed, the chloroplast moves over to the side wall, the protoplasm contracts, and globules of various sizes begin to appear (figs. 2–5). These globules did not turn black when tested with osmic acid, nor purple when tested with iodine. The chemical nature of these globules was not determined, but there is little doubt that they are stored food that may be used by the zoospore and young sporeling. The mass of the zoospore approaches the opening, forming a peak just opposite it (fig. 5). This peak passes into the opening and becomes more and more attenuated, until it extends completely through the opening.

The part on the outside begins to enlarge as currents of cytoplasm begin to pass through; and the part on the outside increases as that on the inside decreases. The cytoplasm showed currents passing through all the time. The chloroplast passed through toward the last, and soon after that all the cytoplasm slipped through the opening (figs. 6–8). Sometimes the opening is ruptured when the zoospore escapes, but more often it remains intact.

One zoospore whose escape was observed came through in the normal manner, until only a small part of the cytoplasm remained in the cell. The part outside seemed to exert considerable force, while that on the inside failed to slip through easily. The connecting cytoplasm was stretched out into a fine strand until finally, as the small bit slipped through the opening, the strand snapped and the small part was lost to the zoospore (figs. 14, 15). The spore seemed to be uninjured, for after about 20 minutes the cilia appeared and it swam away.

When the thallus is two cells thick the zoospore from the outer cell may escape, and the one from the lower may follow soon after. The contents of the lower cell round up and form the globules just as in the upper (fig. 9). Some kind of pressure seems to be exerted upon the cross wall, which gradually stretches. The spore thus pushes farther and farther into the upper cell, until finally the wall snaps and the zoospore is freed in the upper cell, from which it escapes as did the one originally formed there (figs. 10–12).

Whether an inner thin wall is always formed around the cell contents, closing the opening, or whether it is formed only at times was not determined. All sections through zoospores just before escaping show the opening to be so closed. However, these are either never formed in some cells, are torn off in the escape of the spore, or are torn away in sectioning. Probably the last is the explanation of the fact that often one finds two adjacent cells, one with and one without the remnants of the thin wall and the old cross wall (fig. 12). At times an empty cell showed this thin wall pushed through the pore and open at the end (fig. 13). The lower cell did not always form a zoospore at once, and when this was the case the cross wall became nearly as thick as the outside one.

TIME OF FORMATION AND ESCAPE.—There seemed to be no regularity in zoospore formation. In some cases patches of cells were found empty, while in others only a cell here and there had formed a zoospore. As a usual thing only an occasional cell forms a zoospore in the younger thalli, while those thalli that have passed through the winter, in or under the ice, are almost completely deprived of cell contents within a very short time after being brought into the laboratory. Great numbers of adjacent cells form zoospores in a single day, while within a week the thallus would hardly have a cell with its contents remaining. Strange to say, however, not all thalli do this, and weeks after being brought in there were thalli with only a few empty cells. After several days of rapid zoospore formation there gradually comes a lull, and later this is followed by another period of rapid formation. No doubt the change of conditions causes the period of rapid formation, immediately following the change from outdoors to the laboratory.

The time taken for the escape of the zoospore, from the time it starts through until it becomes free, varies from a few minutes to an hour. After the spore becomes free it remains quiescent for 5–20 minutes. At times it rolls over and over in place, and there appears to be some struggle going on; soon the cilia can be seen beginning to wave. First one and then the other begins to move.

The zoospores begin to appear about 7:00 A.M. and continue to escape until about noon. The greater number usually escape between 9:00 and 10:00 A.M. Material brought into the laboratory one morning seldom fails to give abundant zoospores by the next morning, fewer the following day, and so on until zoospore formation almost ceases.

SPORE.—The form of the zoospore varies from spherical to egg-shaped (figs. 16, 17); and is the same for all the species studied, save that those formed from the larger cells of *C. scutata* are inclined to be longer and more slender than the others. The size of the spore varies with the size of the cell and with the species to which it belongs. The platelike chloroplast seems to be able to assume any form, for when it occupies the large end of the egglike spore it is cup-shaped; when on the side it is only slightly cupped; while in its normal position in

the cell it is more or less flat. From this one would infer that the plastid lacked rigidity and very definite form, although in general the platelike form is maintained. The chloroplast shows dense and less dense areas, which give it the appearance of being vacuolate (fig. 16). The pyrenoid is not visible in any of the spores studied. Other evidences of the lack of prominence in pyrenoids when the plastid is not functioning rapidly were obtained. It seems quite probable that the pyrenoid loses some of its density when not functioning, and also that the absence of starch grains around it may fail to throw it into such bold relief as to make it noticeable. The vacuolate structure and lack of prominence of the pyrenoid were observed in living material, and also in that killed with osmic acid fumes but not stained. The globules observed in the developing zoospore are likewise to be found here.

The cilia are two in number and of approximately equal length. They seem to arise from the same spot, or at least to leave the membrane at the same place. How they originated was not determined, because of the inability to catch the developing zoospore as it lay outside the pore of the cell from which it came. There is no doubt that the cilia arise during the quiescent period just outside the pore. In all probability they arise from a granule or blepharoplast, although this could not be proved definitely. In order to prove this, some method must be devised by which the spore during the quiescent period can be killed, stained, and sectioned. Granules of various kinds, possibly chondriosomes, were observed at all the stages of spore formation. Many of these were large and dense, as shown by the deep stain. If a blepharoplast is present it could not be distinguished from these granules (figs. 5–11). The cilia are quite heavy for cilia, and are from two to three times the length or diameter of the spore.

ZOOSPORE MOVEMENT.—The zoospore moves slowly at first, gradually increasing in speed, although at no time does it move as rapidly as the spores of some other algae. The zoospores move forward almost in a straight line, rotating on the long axis all the time. After rotating in one direction for a time they reverse the direction, and this change invariably comes when the chloroplast side is down. Sometimes the zoospore spins round for a brief time, the cilia seeming

to be entangled; and, after a few seconds, it rights itself and swims away.

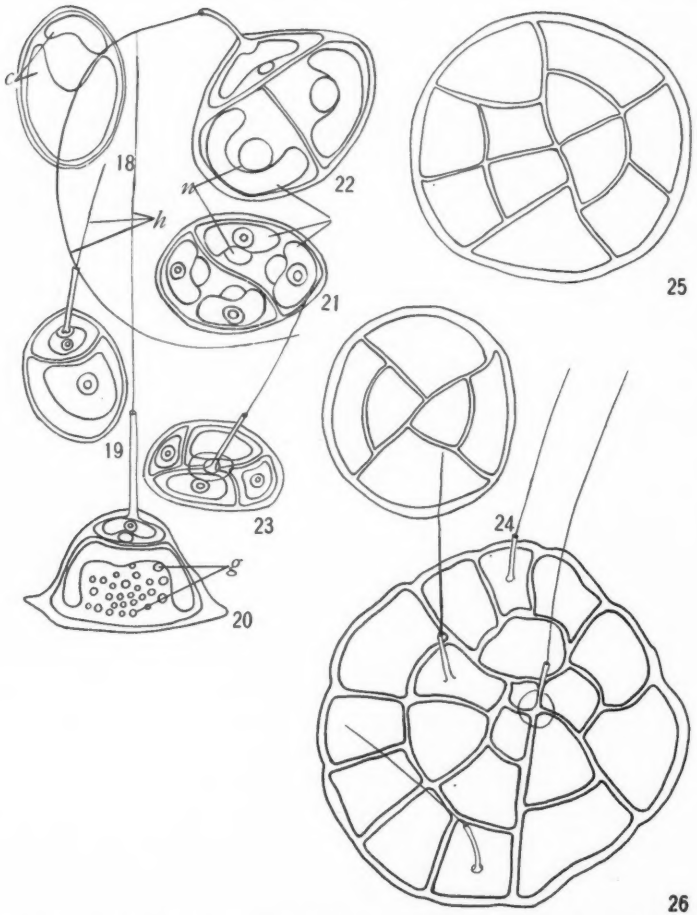
After swimming from an hour to an hour and fifteen minutes, the movement becomes more and more sluggish, and the spore seems unable to manage its cilia very well. They often become entangled, and sometimes one of them could be seen trailing behind the spore. Finally the spore comes to rest with the somewhat larger end down. The cilia are lost, and the wall thickens.

Development of plant body

EARLY STAGES IN C. SCUTATA.—After the thickening of the wall of the zoospore, the chloroplast divides in the usual manner into unequal parts; and likewise the cell divides into an upper smaller and a lower larger cell. The wall is laid down parallel to the substratum. The outer and smaller cell soon forms a hair, and after that never divides or develops any more (figs. 15–20). These changes usually although not always take place in the first twenty-four hours. Next the basal cell becomes very much enlarged and the second division occurs. This wall usually consists of two curves turned in opposite directions, and extends through the long axis of the cell (fig. 21). The depth of the curve varies, while the line of division also varies through an angle of 45°, but nearly always the line passes through the long axis of the cell. At this stage part of the globules are still visible, although before the next wall is completed they have disappeared.

The two cells thus formed may divide simultaneously, or they may divide one at a time. The walls may come in opposite each other, or they may divide unequally, with the small cell of one opposite the large cell of the other (figs. 23, 24).

LATER STAGES IN C. SCUTATA.—After the thallus has attained four cells in one plane and has enlarged considerably, cell division continues. The next divisions may be tangential or radial (figs. 24, 25); probably they are more often radial than tangential. If the first are radial and the second also radial, nearly always the next will be tangential. After these first divisions tangential and radial divisions continue, but by no means do they alternate as is shown in fig. 26. Although the divisions are not simultaneous, cell lineage can be traced accurately. As soon as a cell becomes internal, in the sense



FIGS. 18–26.—Fig. 18, zoospore showing division of chloroplast into small upper and large lower ones; fig. 19, two-celled sporeling before growth of lower cell; fig. 20, same after growth: *gl*, globules; *h*, hair; fig. 21, three-celled stage from above, showing lower cells with chloroplasts and nuclei divided ready for formation of new walls; fig. 22, three-celled sporeling from the side, or possibly a five-celled one: *c*, chloroplasts; *n*, nucleus; fig. 23, five-celled sporeling showing large and small cell formed from each of basal cells, dorsal view; fig. 24, sporeling showing that fourth division sometimes forms tangential walls; fig. 25, young thallus showing fourth divisions tangential on one side and radial on other; fig. 26, later stage in body formation, showing relative number and distribution of hairs, also irregular cell division.

that it forms no part of the periphery, cell division ceases; and these cells never divide again unless under the stimulus of reproduction or as a result of injury. Enlargement of the thallus is due entirely to the division of peripheral cells.

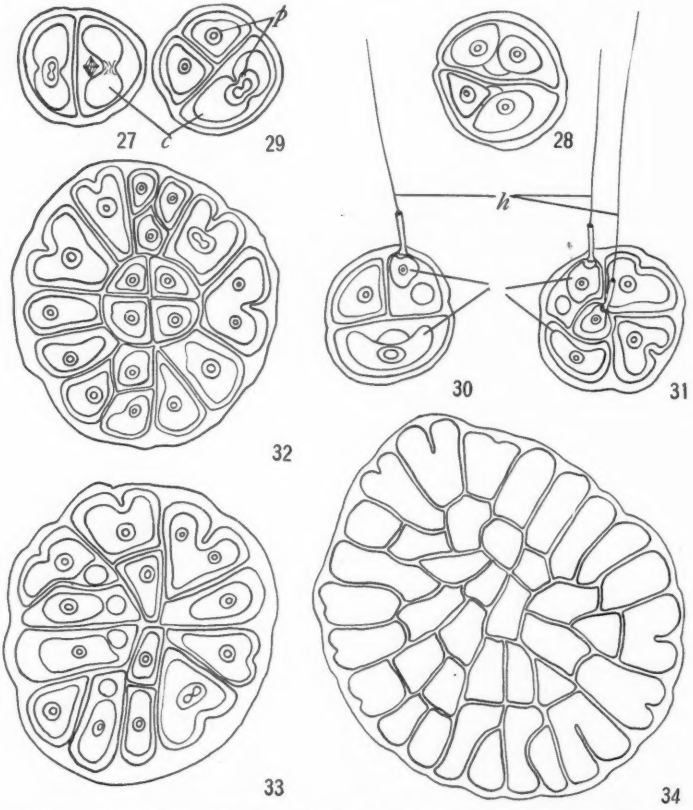
STAGES IN C. ORBICULARIS AND C. SOLUTA.—The behavior of the zoospores is as described for the entire genus. The first wall laid down in the development of the sporeling is perpendicular to the substratum, and divides the spore into two equal parts (figs. 27, 28). The next two divisions are usually at the same time and at right angles to the first; or sometimes they occur at different times and cut off cells unequal in size (figs. 28–31). So far the sporelings coming from the two species cannot be distinguished.

Even at the three-celled stage hairs may begin to appear, and in *C. soluta* they arise from the lateral wall rather than the dorsal, as is the case in *C. orbicularis* (figs. 30, 31). The peculiar method of cell division, which so clearly distinguishes *C. soluta* from the other species, sometimes begins to show as soon as the sporeling has attained the four-celled stage (fig. 30). In this species the wall, instead of being formed at the time of nuclear division and along the line of the cell plate, as is the usual method, arises as an outgrowth from the peripheral wall, and follows the line of the dividing chloroplast. This chloroplast at such a time often assumes the form, more or less, of a heavy Y-shaped body. The first division in a cell with this kind of a chloroplast usually cuts off one of the upper prongs of the Y, while the following one cuts off the other. In this way not only is the number of rows increased but also the number of cells in the row (figs. 32–34).

In all the thalli of *C. soluta* studied the first and inner cells are closely pressed against each other, and so far as appearance of the relation of cell to cell goes could not be distinguished from those of *C. scutata*. Some of the older thalli showed a very slight space between the filaments making up the thallus, and from the periphery there were short irregular extensions of the thallus, but nothing giving the filamentous character.

In *C. orbicularis* the arrangement of the cells and the method of division are about the same as in *C. scutata*, but the size of the cell is much reduced. The cells divide much more irregularly than in

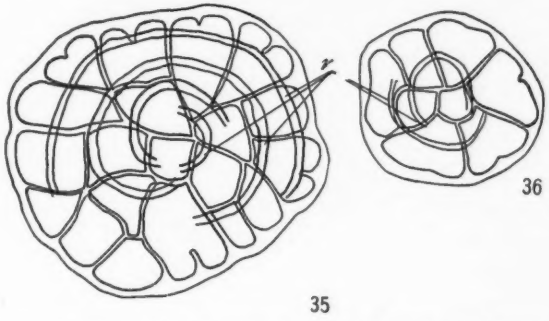
C. scutata, hence the thallus resulting therefrom has the cells more irregular in arrangement and also in form. The mature thallus has a rather regular outline as compared with *C. soluta*.



FIGS. 27–34.—*C. soluta* and *C. orbicularis:* figs. 27, 28, two-celled sporelings preparing for second division, and showing nucleus, chloroplasts, and pyrenoids in different stages of division; fig. 29, three-celled stage but showing the other one of first two cells of sporeling in preparation for division. *C. soluta:* fig. 30, three-celled sporeling showing lateral placing of hair; fig. 31, five-celled sporelings showing method of wall formation characteristic of this species, also placing of hairs; fig. 32, young thallus which has arisen from four equal cells formed from zoospore; fig. 33, young thallus formed from four unequal cells developed by division of sporeling; fig. 34, larger thallus showing every stage in cell division, from merist indentation to completed wall cutting off one prong of Y-shaped chloroplast.

As growth proceeds the younger thalli leave broken, more or less regular rings on the surface. These seem to mark the outer limit of successive periods of growth (figs. 35, 36). Whether these lines are purely mucilaginous, or partly or entirely cellulose was not determined. They have the same appearance and react to the stain in the same way as do the cell walls.

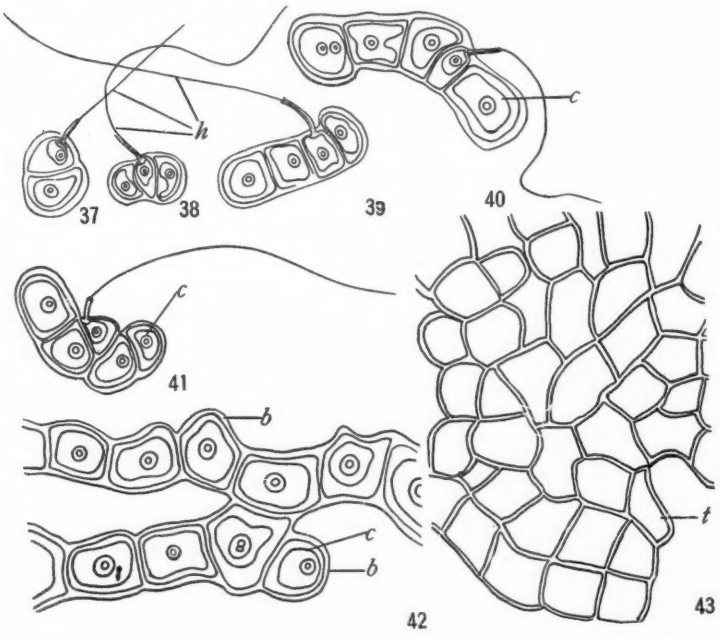
STAGES OF C. IRREGULARIS.—The zoospores of *C. irregularis* divide in the same manner as those of *C. soluta* and *C. orbicularis*. Each of the two cells enlarges and divides simultaneously, or at



FIGS. 35, 36.—Young thalli showing rings marking limits of different stages of growth.

different times, or one of them may form a hair before division takes place (fig. 37). More often one cell divides and cuts a small cell which forms a hair and never divides after that, reminding one of the upper cell in *C. scutata* (fig. 38). Fig. 41 shows one with an upper cell bearing a hair, but this was not formed as it is in *C. scutata;* however, the end result was just the same. After the sporeling becomes three-celled, the end cells elongate and divide and the new end cells do likewise, continuing the growth of the filament (figs. 39–41). Fig. 42 shows different stages in the formation of the branches. Any free wall may bend outward and continue to bulge, until it has formed an extension almost as large as a cell. At the same time the chloroplast grows out into this and finally divides along the line of the old plastid, after which the nucleus divides and a new wall cuts off the branch cell, which acts just as any end cell would. Branching is so profuse that the entire surface of the substratum often becomes cov-

ered with cells (fig. 43); in fact, every free surface seems to tend to branch, and the end result in an old plant is a platelike mass showing no tendency to a morphological center. From the outer edge



FIGS. 37–43.—*C. irregularis:* fig. 37, two-celled sporeling showing one with hair (*h*); fig. 38, three-celled sporeling showing center one with hair (*h*); *c*, chloroplast; fig 39, four-celled sporeling showing one enlarged end cell and one cell with hair; fig. 40, five-celled sporeling showing end cells enlarged and getting ready for division; fig. 41, five-celled sporeling showing production of one hair bearing cell which never developed any more, and the other four cells developing from one of the two first cells of sporeling; fig. 42, young plant showing different stages in branching; *b*, branches; fig. 43; platelike plant body, developed by profuse branching; *t*, thallus.

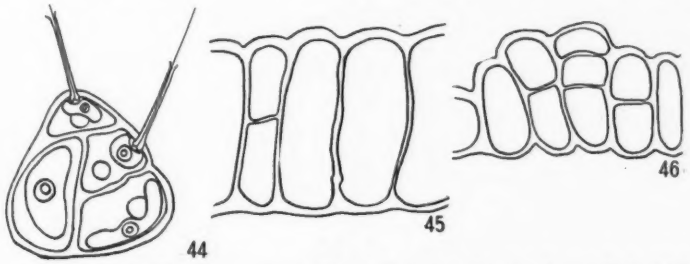
much branched filaments extend in every direction, giving the whole a different appearance from the disk type.

All the species studied save *C. irregularis* may become two or three cells thick, and especially is this true where the thallus grows over an irregular surface. Even very early in the development from the spore this increase in thickness may appear (fig. 44). There

seems to be no regularity to the division of cells parallel to the substratum. Only one cell of a large group may divide, or only one may fail to divide (fig. 45); then again an equal number may divide. More than two cells are seldom formed unless the thallus is growing over a very irregular surface. Fig. 46 shows a thallus three cells thick on a more or less regular surface.

Evolution and development of plant body

In the case of *Coleochaete pulvinata* and *C. divergens*, the plants are distinctly filamentous with no tendency to form a plate. In *C. irregularis*, while resembling the other two in being filamentous and



FIGS. 44–46.—Fig. 44; young sporeling of *C. scutata*, showing three cells in thickness, one of which is first cell cut off by spore; fig. 45, section through *C. scutata* showing one cell that has divided parallel to substratum, surrounding ones remaining undivided; fig. 46, section of *C. orbicularis*, showing three cells in thickness formed on regular substratum.

branched, the mature plant shows a distinct tendency to be platelike, due to profuse branching. There is no morphological center, however, and cell sequence cannot be worked out, but when this is separated from the thing on which it is growing, it holds together in one mass. This plate effect is attained by means of branching, a branch being put forth from every free wall. In *C. soluta*, on the other hand, the branching is attained by a division of an end cell. At first the thallus shows no sign of being filamentous, while later spaces are to be found between the lines of cells; and at the periphery the lines may have very unequal growth, and the outline may be quite irregular. In *C. orbicularis* and *C. scutata* the plant body seems to have become definitely platelike. The growth shows little or no tendency toward the filamentous form, save that growth is

terminal, and that new rows of cells are made by division of the end cell, rather than branching from an old cell. Because of these, the disk might be interpreted as a branched filamentous form that appeared platelike, because it was held close against the substratum by the attaching fibers and because of the profuse branching.

Hair formation

FORMATION OF OPENING.—The opening in the wall through which the hair extends seems to be formed in exactly the same way as that of the opening for the escape of the spore. At any rate, when there is definite indication that a hair is to be formed the pore is already there; and it appears to have been formed in just the same way. Of course, if it were formed in just the same way, one could not tell whether the cell is to form a spore or a hair, save that the opening for a hair is slightly smaller than that for the escape of a spore.

EARLY STAGES.—Soon after the opening is formed, or at about the time of its completion, a cell wall is formed just inside the old one. The chloroplast rolls into an incomplete cylinder, with one of the open ends just below the opening in the wall. About this time a dark deeply staining egg-shaped granule is seen, either in the lower part of the opening or in the upper open end of the chloroplast (fig. 47). This granule is suspended in the open end of the chloroplast by means of cytoplasmic strands, at least at times, although they were not always in evidence. Quite often a second and larger granule is found lower down in the space inclosed by the chloroplast, and associated usually with the nucleus. A heavy strand of cytoplasm connects these two granules. Sometimes other granules, less egg-shaped and more nearly round, are seen in the cytoplasm, but always the one, and sometimes the two, are so definitely formed and of such a uniform size and density as to make them quite conspicuous. In the unstained material these showed plainly as highly refractive granules.

HAIR FORMATION PROPER.—From the granule in the opening a dense, finely granular stream seems to issue. This was caught in different stages, showing the stream just beginning, just entering the opening, and then extending into the opening or nearly through it (figs. 48–51).

SHEATH.—At this time the cone of the sheath is clearly visible. The cell wall formed just before the pore was complete seems to be pushed up into the pore. From the side this gives a conelike outline. It is still in a very plastic state. The stream of cytoplasm seemed to force it into the opening, or perhaps pressure of the cell contents did this; at any rate it extended into the pore and the stream of granules extended through the center, leaving considerable space between the sheath and itself (figs. 48–50). The sheath is forced farther and farther, until it extends for some distance beyond the limits of the wall. As the sheath increases in length the base becomes much enlarged, and extends into the cell. This enlarged part forms an extension of the newly formed wall. At this time the entire sheath becomes thicker, and reacts to the stain in the same way that the normal wall does. When completed the enlarged knoblike base shows an inverted funnel-like opening through which the granular stream passes (fig. 54). The upper end of the chloroplast wraps round this enlarged base.

HAIR.—Finally the developing sheath is ruptured at the end, and the finely granular stream continues out into the water. The finely granular cytoplasm may become homogeneous before or after emerging from the sheath (fig. 55). Just what brings about this change was not determined. It may be due to contact with the water, or it may be due to some change within itself. At any rate the hair continues to grow until it has become 3–8 times the length of the sheath (figs. 20, 22).

Usually the hairs project at right angles to the surface. Sometimes, however, immediately after leaving the pore they curve in such a way as to lie flat against the surface of the thallus. Again they may curve so much and so tightly as to form a knot (fig. 57). In a thallus in which the zoospores had escaped from the outer cell, while the one from the lower had not followed, the cross wall became thickened, and the lower cell formed a hair in the usual way (fig. 58). The sheath of this extended through the pore in the wall of the outer cell. A case similar to this was one in which the adjacent cell to one from which a zoospore had escaped formed a hair with the sheath extending into the empty cells. Only two cases of this kind have been observed, and both were killed before the sheath was complete.

What the course of the hair would have been can only be conjectured, but in all probability it would have grown through the pore in the outer wall (fig. 59). Another very peculiar formation was the sheath showing four hairs coming from it (fig. 61). One was found with two and another with three hairs. An abnormal case was one with two granules side by side (fig. 60). The cytoplasmic streams united, and this may be the explanation of the two to four hairs coming from one sheath.

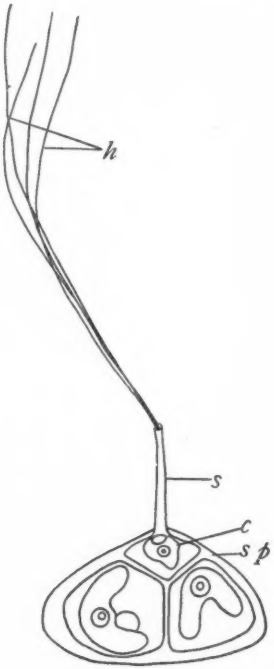


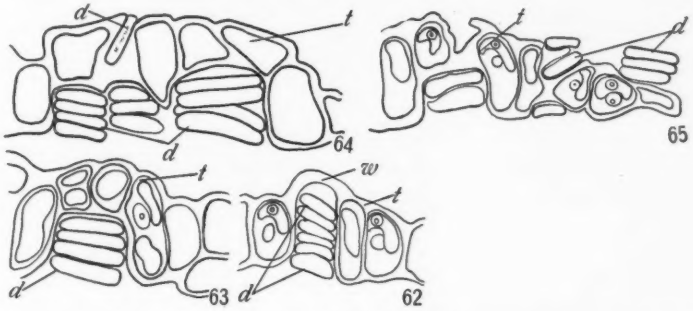
FIG. 61.—Four hairs coming from one sheath; *c*, chloroplast; *h*, hairs; *s*, sheath; *sp*, sporeling.

Relation to diatoms and substratum

RELATION TO DIATOMS.—No instance of the germination of a zoospore on top of a diatom was found, but often the zoospore germinated near them and grew over them. Sometimes there is only one, and at other times there are 4–6 piled one above the other underneath the thallus (figs. 62–65). Usually smaller cells are formed above these, but one was found in which no sign of a cell above the diatoms could be seen. There appeared to be only an extension of the walls over them (fig. 62 *w*). The thallus shows a remarkable ability to adapt itself to the irregularities of the surface over which it is growing. Diatoms were not only beneath the thallus, but in such great numbers on its top that a surface view of the condition of the thallus was impossible at times. Sections through the plant revealed diatoms almost completely inclosed in the tissue (figs. 64, 65).

ATTACHMENT TO SUBSTRATUM.—Soon after the zoospore comes to rest and begins the formation of its cellulose wall, fiber-like projections begin to form, extending from the wall in the direction of

the surface on which it is resting (fig. 66). In the three-celled stage of the sporeling the fibers seem to grow toward the lateral walls of the epidermal cells, while an older, more mature thallus shows these attaching organs growing down between the lateral walls (figs. 67, 68). Whether these fibers always penetrate between the lateral walls of the epidermis was not determined. One that grew between the guard cells of the stomata was much thickened before it finally reached the wall where it was attached (fig. 69 *w*). It seems quite possible that lack of pressure may have stimulated the cells to produce these, for there is a slight depression where each lateral wall is



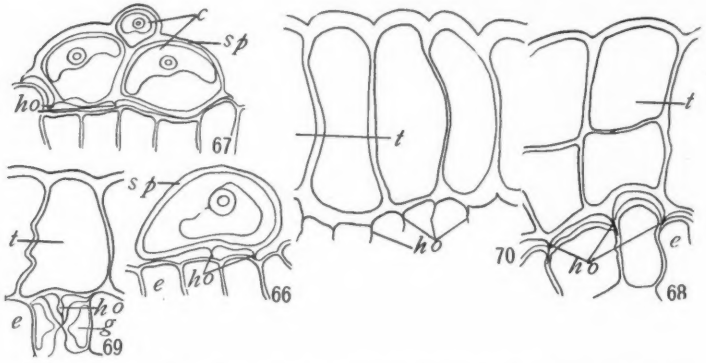
FIGS. 62–65.—Fig. 62, cell wall covering group of diatoms; *d*, diatoms; *t*, thallus; *w*, wall; figs. 63, 64, diatoms underneath thallus; fig. 65, diatoms on thallus and almost inclosed by it.

found. That this method of attachment is quite effective is shown by the force required to separate the thalli from the substratum. One can wipe off the surface with considerable roughness and pressure and make no impression on them. The thalli tear into pieces more easily than separate from the substratum. Fig. 70 shows a portion of a thallus that had grown over diatoms. It had been torn away, leaving the fibers attached to the lower walls. Whether this is the method of attachment to substances like glass was not determined.

Aplanospore production

In all the species studied aplanospore-like cells are formed. The contents of a cell seem to have contracted and formed a heavy wall around the entire mass. Inside the wall the cell contents are quite

dense, and filled with small globules much like those in the zoospore but smaller. These are obviously stored food. No chloroplast could be found in any of the sections or whole mounts studied, but thalli from which all the other cell contents have escaped have an occasional aplanospore, which shows bright green, although here again the chloroplast could not be seen (figs. 71, 72). It is possible that these sporelike bodies are other plants that have gained entrance into the cell, but they were found in such abundance in some thalli



FIGS. 66–70.—Figs. 66, 67, one- and three-celled sporelings, showing early development of fiber-like holdfasts; *e*, epidermis of *Sagittaria; ho*, holdfast; *sp*, sporeling; fig. 68, portion of thallus showing holdfasts penetrating between lateral walls of epidermis; fig. 69, holdfast developed between guard cells; *g*, guard cells; fig. 70, portion of thallus that developed over diatoms and put out holdfasts between (cutting separated it and revealed the long holdfasts).

that this explanation hardly seems probable. However, these bodies were not germinated, and therefore no definite statement can be made.

Regeneration

By regeneration is meant here a rejuvenation of the cells around an injured area resulting in the formation of new lobes of the thallus. While *Coleochaete* when injured does not, as do some animals, form new parts to take the place of those removed by injury, it does form new tissue around the injured region. Injury to the thallus of any of the species is quite common, and probably the commonest cause of injury is the snail. These are found in every stage of development,

from the egg to the adult, wherever *Coleochaete* is growing. The contents of any injured cell may either disintegrate within the old cell wall, be diffused into the water, or be consumed as food by the snail. In any case the adjacent cell walls would be exposed to outside influences, and immediately begin to thicken (fig. 73).

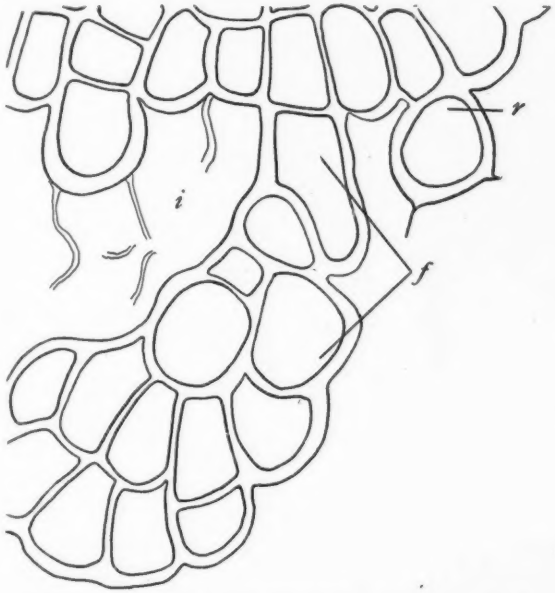


FIG. 74.—Fan-shaped growth developed from rejuvenated cell

Any number of cells around the injured area might show an increasing density and a deeper green color. The exposed walls, as soon as the adjacent cells were removed by injury, bulged out. The walls continue to increase in size, and cell division follows (fig. 71 *r*). Sometimes only one cell in a place is rejuvenated, while in other places two and even three cells may form the new lobe of the thallus, all three acting in the same way as any three peripheral cells of an uninjured thallus. The method of division and the general appearance of the lobe correspond to that of a portion developed from one to three of the cells of a sporeling. Here, as in the case of normal

growth, new cells are always formed by the division of peripheral cells (fig. 74).

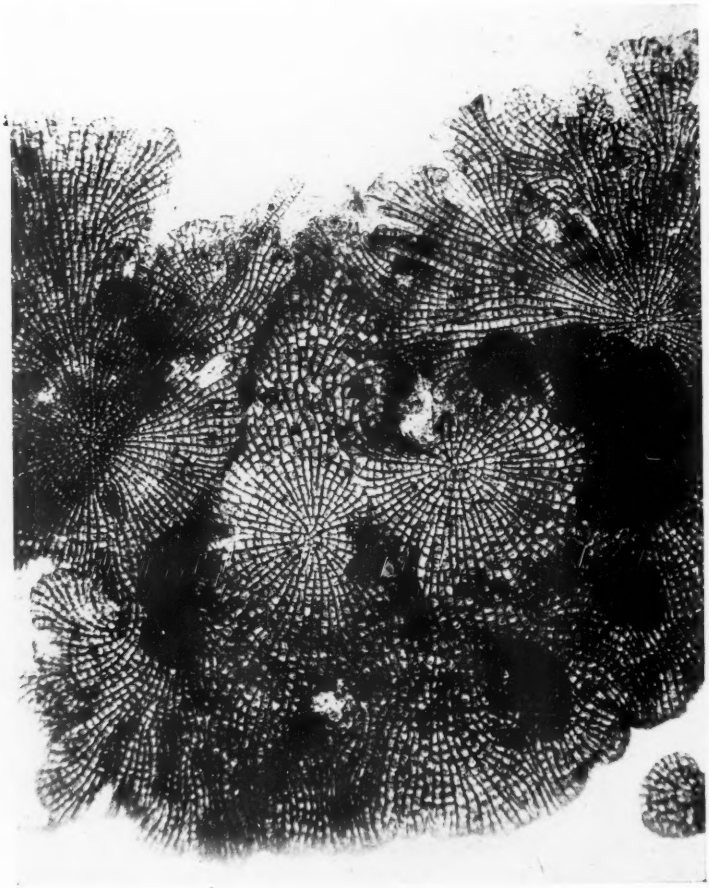


FIG. 75.—Photomicrograph showing ruffled and fluted edge

The species used to illustrate this phase was *C. scutata*, but the general behavior is the same for all the species studied. One thallus observed showed rows of cells 4–6 cells in length, each row being

separated from the other by about the amount of space that some 2–4 cells would occupy. This region was near the center of the thallus and covered about one-half of it. By the bulging thick walls these rows of cells showed that they had been produced by rejuvenation. Some four or five tangential divisions must have preceded the first radial one.

After radial division begins, the new growth follows the normal habit of growth, until soon the cells have grown out and pressed against one another. From this point the thallus has the appearance of any other normal one.

Because of the lack of lateral pressure the new cells spread and continue to spread, until the new part becomes fanlike. In those plants that are injured around the periphery after they have reached maturity, the numerous outgrowths give them the appearance of being ruffled or fluted (fig. 75).

A peculiar thing about this new tissue is its tendency to grow, not only over the old uninjured portion, but also over any other new lobe produced in the same way. In contrast with this, thalli developed from two zoospores that had settled down near each other, form cells that meet in the process of growth and simply flatten their ends against each other; and the next ones do likewise, until the cells are so closely associated that they cannot be distinguished from any two cells of the same thallus. The fact that two zoospores, instead of one, are responsible for the formation of the plate of cells can only be seen because of the peculiar angle at which the cells meet and the two morphological centers. When separated from the substratum the thalli break apart at any other place as easily as along the line of union.

Another interesting thing to be noted in this connection is that when fruiting begins in two thalli that have grown together in this way, the first antheridia are always formed just back of the line of contact of the two.

Discussion

The work of PRINGSHEIM on five of the nine species of this genus laid down in a remarkably clear and accurate manner the broad outlines of the genus. In spite of his excellent work, however, some of the finer details were omitted. PRINGSHEIM (13) described accurate-

ly the change in position of the chloroplast in zoospore formation, also the appearance of the globules or droplets. As to the actual manner of escape of the spore, he merely noted that it escaped through either a break in the wall or a pore. The formation of the pore prior to the change in position of the chloroplast was not noted. This pore was evidently the result of some chemical change brought about by the chloroplast. More than likely it was made by an enzyme produced by the chloroplast. The appearance of the change, as well as the outline of the region of change, seemed to point to this.

A very interesting question was why the spore forced itself through the narrow pore. According to JOST (5) the zoospore escapes because of the swelling of the inner membrane. He also claims that they will not escape if kept in the dark, and reports that the first division of the zoospore which had been kept in the dark occurred without shedding. Nothing in any of the spores studied indicated a swelling of a sheath or anything of the kind, while the effect of light was not tried. The shrinkage of the cell contents seems to preclude the idea of pressure from within, while no cause for a lessening of pressure from without could be found. The spore passed through by an amoeboid movement, and probably went through the opening because that was the only place where it was not stopped by the cell wall. It seems quite probable that the movement was entirely without direction, save for the light stimulus that JOST speaks of. The movement of an amoeboid mass across a glass slide when mounted in pure water must be purely aimless. It will crowd between any obstacles in its way in the same manner that the spore goes through the pore. Although the spore does not put out pseudopodia, save the extension that takes it through the opening, it seems probable that its passage is quite similar.

The only reference to the time of production of zoospores is that by JOST, who says that they escape in the early morning hours. No one gave the time, or behavior of the young spore, hence a record of the exact time as well as the early behavior was made.

The form, light refracting globules, and the number and position of the cilia observed by former investigators were confirmed by this work. The only point of departure of the two accounts consists in the description of the chloroplast as given here. The movement of

the spore had been accurately described by PRINGSHEIM and JOST, but some minor details, together with the length of the period of movement, were not given by them, nor did they mention the quiescent period.

Their account of the development of *C. scutata* differs from mine only in one point: the double curved line of the second division by the sporeling, instead of being the exception, or one of the forms of division, as given by JOST, is almost the only kind in the many hundreds observed by the writer. Of course there is quite a wide variation of the angle that this line makes with the long diameter of the cell, but it most often runs through the longest line.

In their accounts of the development of *C. soluta* and *C. orbicularis* there is a wide divergence from this account. They get two cells from the zoospore just as I do, but PRINGSHEIM then gets an outgrowth in the form of a curving branch from each of these two cells, and these branches put out other branches from which the filaments arise, while the material used for this work showed the first two cells enlarging and dividing into two equal (or large and small) cells. Both were found frequently, but not a single instance was found of the kind reported by PRINGSHEIM, in spite of the fact that literally hundreds of germinating zoospores were examined. After the formation of the first four cells, they enlarge and new cells arise by their division. After the second circle of cells is formed, division of the peripheral cells by growth of the wall from the outside in causes the growth of the thallus in the case of *C. soluta*. It is hard to account for this difference, since one could hardly mistake this for any other species. Of course it is possible that this is a variation of the same species, or that the difference in environment causes the variation in development. Such a careful observer as PRINGSHEIM could hardly have been mistaken in what he saw. On the other hand, check and recheck with his results in mind have always yielded the same result.

LAMBERT (**6**) reports that the plant body is 1–3 cells thick, while PRINGSHEIM gives it as one cell in thickness. My observations confirm LAMBERT'S account. Just why the thallus should vary along this line is hard to understand. It cannot be due to growth under pressure, resulting in cells too large to take in food properly, for very

often only one cell will divide parallel to the substratum, while all the surrounding cells will remain undivided, in spite of the fact that they are as long and are often broader than the one dividing. On the other hand, all of a group of cells may divide save one or two. Thalli three cells in thickness are rare; most of those were observed where the plant grew over a very uneven surface.

According to PRINGSHEIM the hair was formed by a pushing out of the wall into a long hollow cylindrical sheath, which finally opened at the end and a bristle protruded therefrom. One end of this bristle remained deeply imbedded in the sheath according to the account. He speaks of separating walls but concludes they are really not cellular hairs. MOEBIUS (9) says that the cell pushes out a thin cone into which the lumen extends. The outer membrane enlarges and detaches itself from the inner. Finally, after a further extension of the sheath, the hair breaks through. This was still surrounded by the inner very tender membrane, and because of its cytoplasmic contents was capable of elongation until it was many times as long as the sheath. According to MOEBIUS the content of the lumen of the hair was homogeneous, and there was no aggregation of cytoplasm at the tip. At intervals along the hair he found small light refracting bodies, but concluded they were not cross walls. He also observed a strong light refracting body at the place of origin of the hair in each of his two Australian species.

As will be seen from this account, there is a wide divergence between their findings and mine. The "strongly light refracting body" of MOEBIUS is, no doubt, the dark staining dense granule from which the hair originates. That, together with the fact that the hair consists of a sheath and hair, are about all there is in common between the two accounts. The facts were given in the body of the paper and need not be repeated here, but a few words of interpretation may be given. That the pore is formed by an enzyme secreted in the same way as in the case of the pore of the zoospore seems entirely plausible, since no sign could be found of the formation of the pore in any other way, and cells were found forming pores as in the case of the zoospores. The pore differs in no way save that it is smaller than the other one.

The dark staining, light refracting bodies are blepharoplasts,

chondriosomes, or granules, as one chooses. These granules, as I shall call them, cause a stream of finely granular cytoplasm to issue therefrom. About this time the increased pressure causes the new very elastic wall to push out into the pore. The stream of cytoplasm goes into the center of this elongating membrane and keeps pace with its development. As this goes into the narrow opening the stream is checked, and there is deposited at the entrance a mass of dense cytoplasm. The cell membrane, because of lack of pressure on the outside and pressure on the inside, continues to extend until it reaches the limit of its elasticity, at which time it bursts, and the stream of cytoplasm which has kept pace with it may pass out into the water, and continue to pass out until it has reached its normal length, which is several times as long as the sheath. Either the water or something else causes the granular cytoplasm to become homogeneous and brittle, and the hair is formed, or the granular cytoplasm merely changes itself. More than likely it is the latter, for often the cytoplasm is seen to take on the hard brittle form before leaving the sheath. At the same time that the hair is forming, the sheath and the wall that formed it are thickening and the base of the sheath is becoming much enlarged and thickened, just as if the cytoplasm had been checked at the entrance to the pore and had deposited cellulose there, and more cellulose, until it extended out into the cell like a delta into a bay. That the cilia are formed by the same body or bodies, and in the same way as the hair itself is formed, seems not entirely improbable, since no sign of cilia could be found in either fresh or stained zoospores until toward the end of the quiescent period after their escape. The spores when observed at this time showed no sign of them, when suddenly they were seen waving in the water. The granules have all the appearances and characteristics of a blepharoplast. In order to prove this, it would be necessary to devise some method to catch these spores at the quiescent stage, and to cut and stain them. So far they have eluded the writer.

Former accounts of the genus have made no attempt to explain how the plant body is attached to the plant or other object upon which it grows; they contented themselves by saying that it is attached.

Under the heading Regeneration, growth after injury was discussed. According to McCallum (8), regeneration is not really different from ordinary plant growth. His work indicates that the growth activity was not stimulated by anything sent out due to wounding, for he found that the growth was started when the parts, instead of being removed, were only prevented from functioning. His conclusion was that a functioning organ in some way inhibited other potential growing parts from functioning. This may explain why growth occurs after injury of the peripheral cells, but cannot explain growth due to injury of internal cells, as those shown in fig. 71. In this case cells that had definitely matured, and that under normal conditions would never grow again unless to develop antheridia, now after death of adjacent cells became active. Only outer cells of this plant ordinarily divide, but any cell around an injured place may begin to divide and form a new fan-shaped part, in whose peripheral cells only rests the power of division. McCallum's reasons certainly do not apply here, since not only are the peripheral cells intact but they are functioning as well. Why then is growth begun here? It cannot be due to release of pressure, because if that were so all the cells would grow, and that is not the case. Why then do some of these cells grow and others do not? Each one has an equal chance for food, water, light, and is exposed to the same temperature. The difference must be in the cells themselves. It is quite probable that all the cells of a thallus of this kind are not in the same physical condition, any more than all the members of the same family are. Those in the better condition recover from the shock of injury, and the lack of pressure on the outside causes the cross walls to push out, due to pressure within. The cell contents increase to keep pace with the increasing space, and growth begins. Since these cells started growing first, they might inhibit the others or might deprive them of available food. This latter does not seem at all plausible, because each cell has equal access to the supplies necessary for growth. Why then do some cells grow while others do not? All peripheral cells grow, and in a way these have all become peripheral. It seems to make no difference whether the cells that grow extend from the peripheral side in, or from the central or lateral sides. Here, if anywhere, the cause of growth following injury should be determined, because here is found about the simplest expression of it.

Summary

1. The opening through which the swarm spore escapes is made by some chemical change, probably enzymatic, which is initiated and controlled by the chloroplast.

2. Zoospores escape by an amoeboid movement through a pore.

3. There is a period of quiescence after the escape of the spore, at the end of which the cilia appear.

4. The second wall in the sporeling of *C. scutata* is almost invariably a double curved line whose openings are turned in opposite directions. This wall passes through the long diameter of the cell.

5. The first two cells of the sporeling of *C. orbicularis* and *C. soluta* divide to form four or six cells. By division of these the thallus is formed.

6. *C. soluta* is developed into a thallus, with no indication of the filamentous character except the small space between the outer cells of the mature thallus.

7. Hair formation is initiated by a stream of cytoplasm issuing from one or two granules, found either in the lower part of the pore or in the outer open end of the cylindrical chloroplast.

8. The sheath is formed by an extension of the new inner wall which is forced through the pore. This sheath develops a knoblike base, around which the chloroplast is wrapped.

9. Thalli are attached to the substratum by small outgrowths from the basal walls.

10. Diatoms may be overgrown by the thallus, they may cover it, or they may be almost completely inclosed by it.

11. Injury may result in fan-shaped portions developed anywhere around the injured region.

This investigation has been carried on under the supervision of Professor CHARLES J. CHAMBERLAIN, to whom I am indebted for valuable advice and criticism. I am also indebted to Professor W. J. G. LAND for helpful advice and the photomicrograph.

UNIVERSITY OF CHICAGO

[*Accepted for publication January 11, 1928*]

LITERATURE CONSULTED

1. ALLEN, C. E., Die Keimung der Zygote bei *Coleochaete*. Ber. Deutsch Bot. Gesells. **23**:285. 1905.
2. BERTHOLD, G., Morphologie und Physiologie der Meeres-Algen.
3. CHODAT, R., Études de biologie lacustre. *Coleochaete pulvinata*. Bull. Herb. Boiss. **6**:457. 1898.
4. FALKENBURG, P., Die Algen in SCHENK's Handbuch der Botanik **2**:249–254. 1882.
5. JOST, L., Beitrage zur Kenntnis der *Coleochaete*. Ber. Deutsch Bot. Gesells. **13**:433–452. 1895.
6. LAMBERT, F. D., An unattached zoosporic form of *Coleochaete*. Tufts Coll. Studies **3**:61. 1910.
7. LEWIS, J. F., Notes on the morphology of *Coleochaete nitellarum*. Johns Hopkins Univ. Circ. N.S. **3**:29–31. 1907.
8. MCCALLUM, W. B., Regeneration in plants I. BOT. GAZ. **40**:97–120. 1904.
9. MOEBIUS, M., Morphologie der haarartigen Organe bei den Algen. Biol. Zentrabl. **12**:71–87; 97–108. 1892.
10. ———, Australian *Coleochaete*. Flora **75**:424–429. 1892.
11. OLTMANNS, FR., Die Entwicklung der Sexualorgane bei *Coleochaete pulvinata*. Flora **85**:1–18. 1898.
12. ———, Über die Cultur-und Lebensbedingungen der Meeresalgen. Jahr. Wis. Bot. **23**:339–440. 1892.
13. PRINGSHEIM, N., Beitrage zur Morphologie und Systematik der Algen. III. Die *Coleochaeten*. Jahrb. Wis. Bot. **2**:1–37. 1860.
14. URSPRUNG, A., Eine optische Erscheinungen *Coleochaete*. Ber. Deutsch Bot. Gesells. **23**:236. 1905.

EXPLANATION OF PLATES I, II

All figures were drawn with the aid of camera lucida Spencer apochromatic objective 3 mm. N.A. 1.40, in combination with compensating ocular ×15, giving a magnification of ×680; except figs. 14–17, which were drawn with Spencer apochromatic objective 1.5 mm. N.A. 1.30 with compensating ocular ×20, giving a magnification of ×1775.

PLATE I

COLEOCHAETE SCUTATA

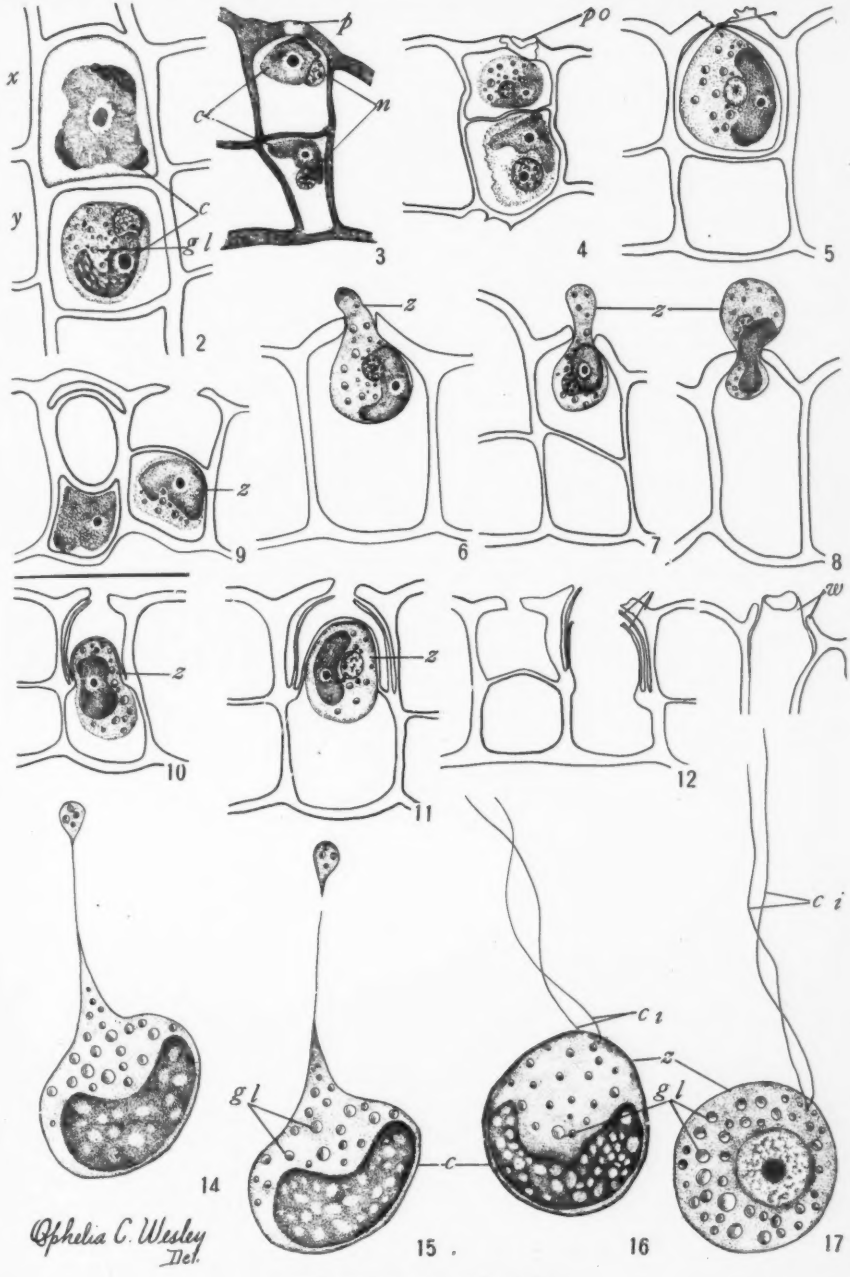
FIG. 2.—Surface view of two cells: upper cell showing platelike chloroplast curled up at corners, otherwise normal; lower cell contents rounded up to form zoospore: *c*, chloroplasts; *gl*, globules.

FIG. 3.—Section through portion of thallus, showing beginning of pore formation: *c*, chloroplast; *n*, nucleus; *po*, light area where pore is formed.

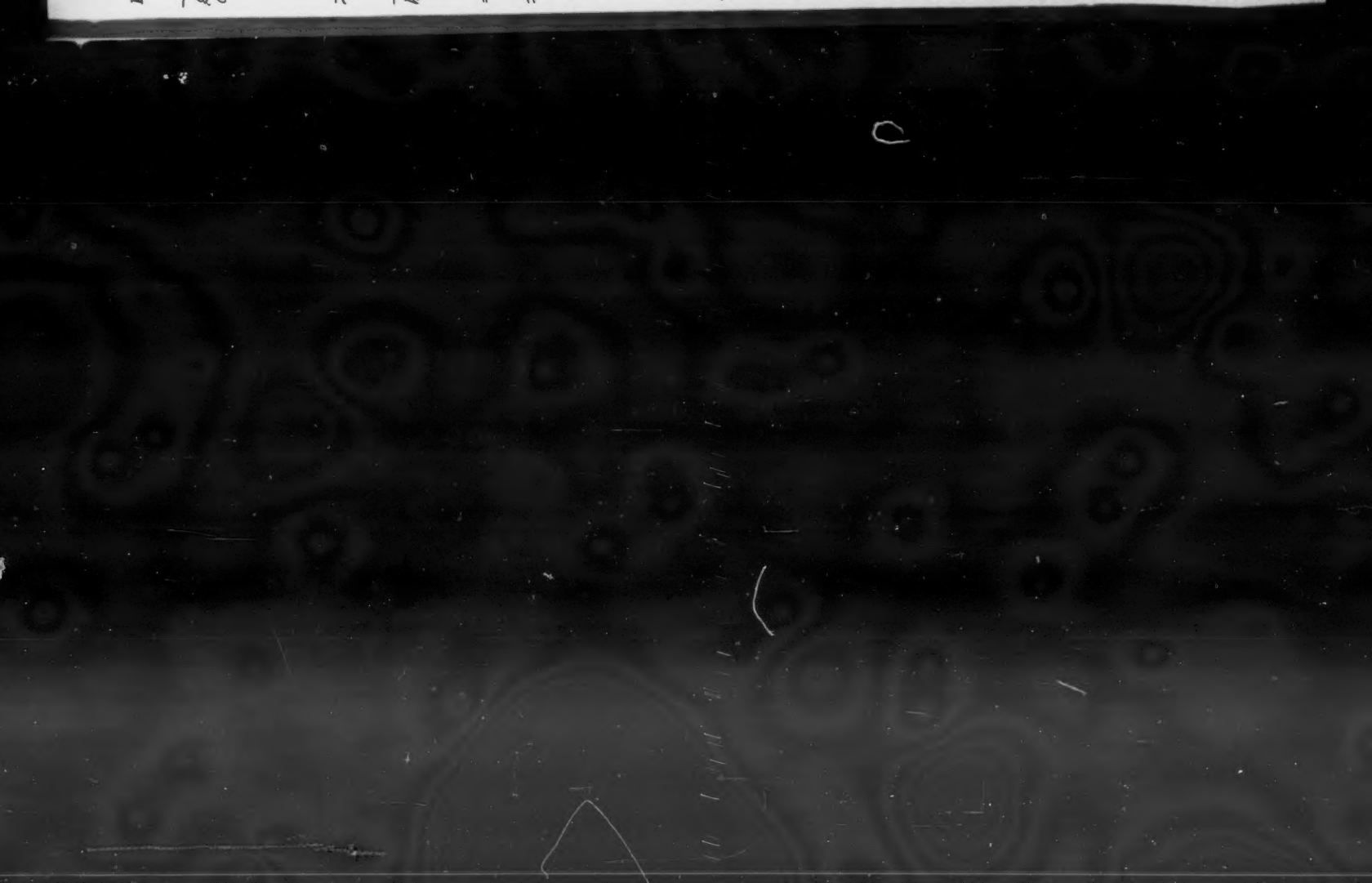
FIG. 4.—Section of thallus, showing completed pore (*po*), cut just to side of pore, showing its regular outline.

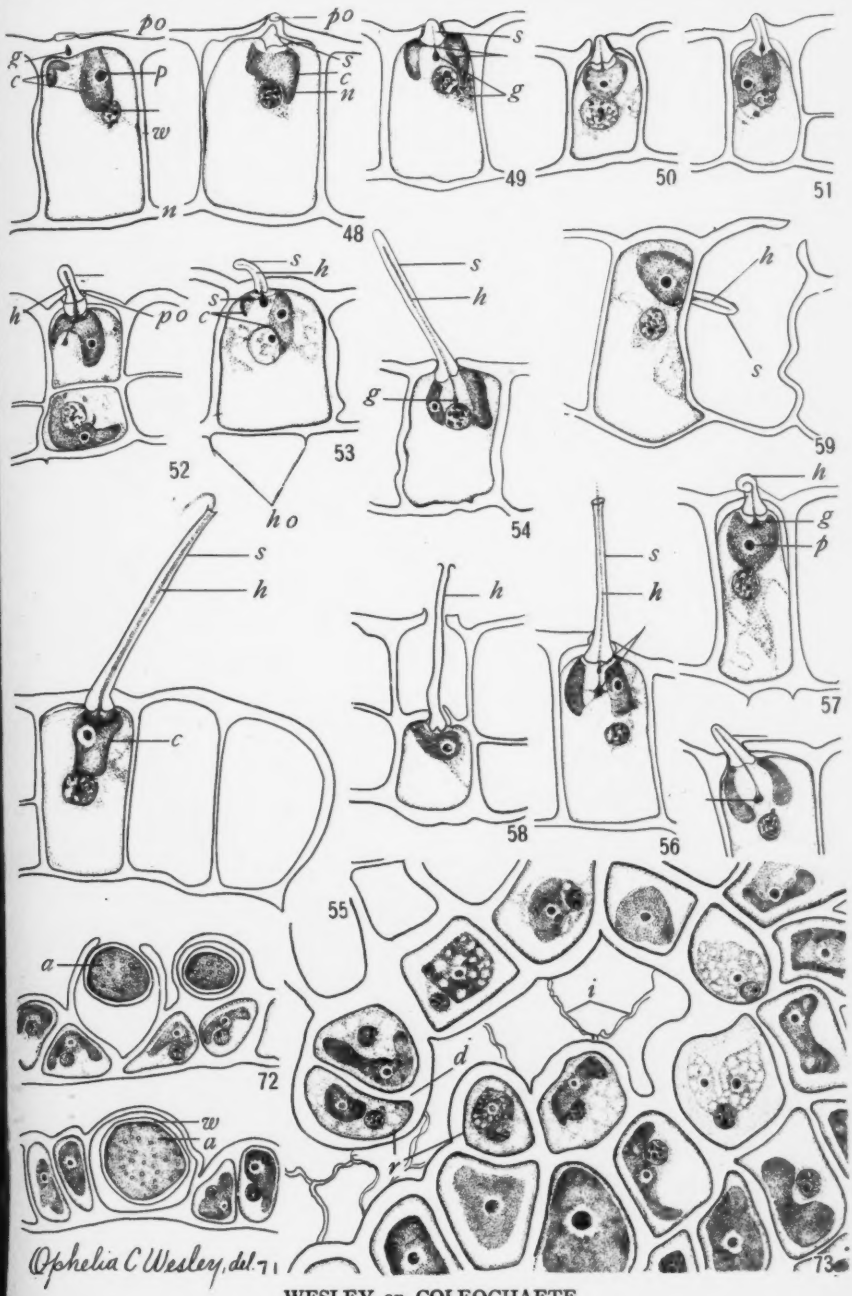
FIG. 5.—Section showing zoospore beaked ready to escape and irregular pore closed by new wall.

FIGS. 6–8.—Stages in escape of zoospore.



WESLEY on COLEOCHAETE





WESLEY on COLEOCHAETE

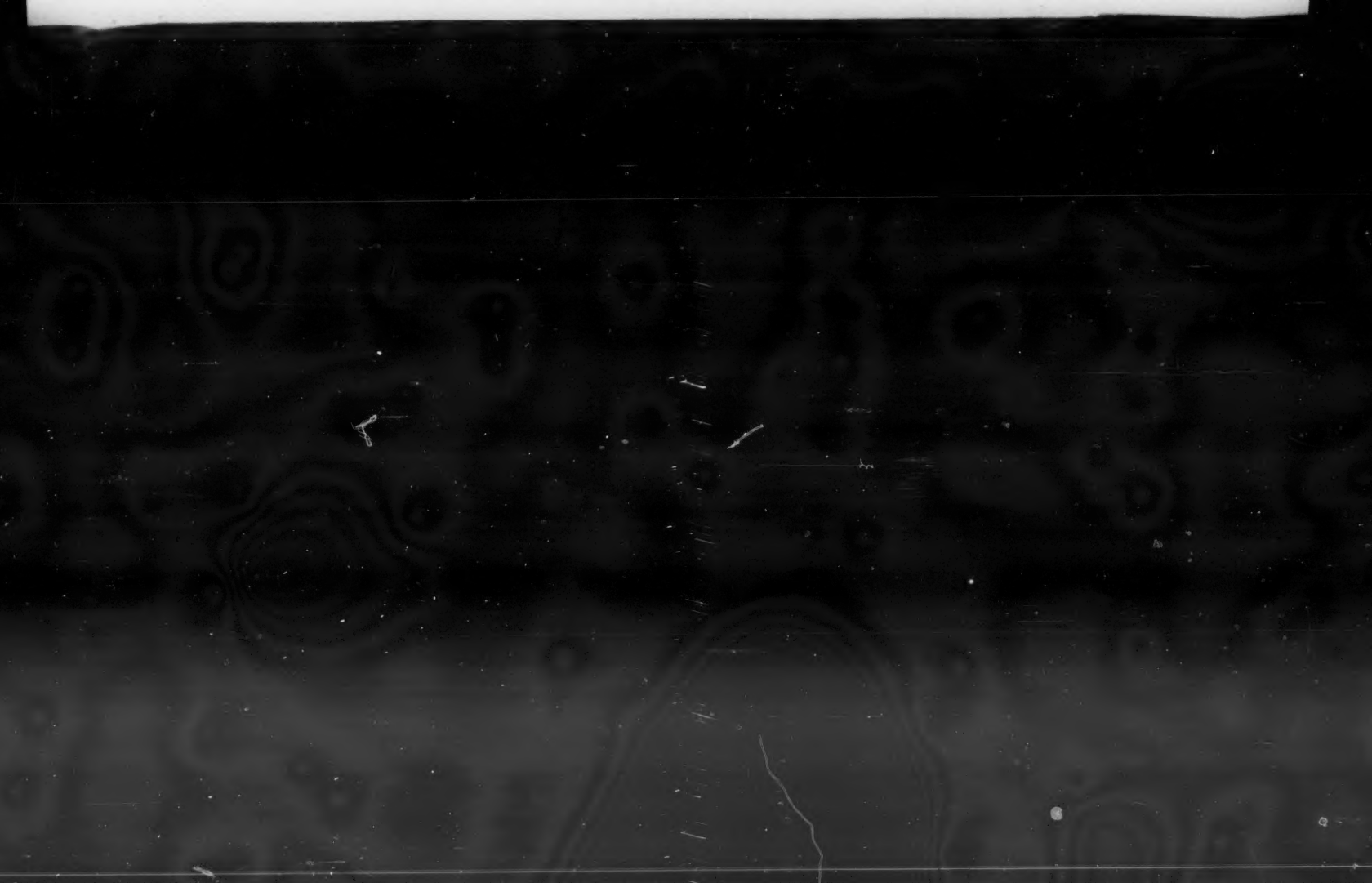


FIG. 9.—Section showing empty cell from which zoospore has escaped and lower cell with contents rounding up to form zoospore.

FIGS. 10, 11.—Stages in escape of spore from lower cell.

FIG. 12.—Cells after escape of spores, showing remnants of new wall, outer one, and inner cross wall which has been much stretched and finally ruptured in escape of the spore from lower cell.

FIG. 13.—Section just to the side of the ruptured new wall, showing it stretched beyond the pore.

FIG. 14.—Escaping zoospore in which the last bit of protoplasm did not slip through pore, and connecting cytoplasm drawn out into long strand.

FIG. 15.—Spore just as the bit of cytoplasm slipped through pore, showing strand snapped in two.

FIG. 16.—Egg-shaped spore: *c*, chloroplast showing dense and less dense areas; *ci*, cilia; *z*, zoospores.

FIG. 17.—Zoospore more nearly round, showing nucleus and not showing chloroplast.

PLATE II

COLEOCHAETE SCUTATA

FIG. 47.—Section through cell, showing beginning of hair formation: *c*, sections of chloroplast; *g*, granule; *n*, nucleus; *p*, pyrenoid; *po*, pore; *w*, wall.

FIG. 48.—Section through cell, showing beginning of sheath formation: *c*, chloroplast in cylindrical form; *n*, nucleus; *po*, pore; *s*, sheath.

FIG. 49.—Section showing beginning of cytoplasmic stream from granule inclosed by chloroplast: *c*, sections of chloroplast; *g*, granules; *s*, sheath.

FIG. 50.—About the same as fig. 49 except that chloroplast not sectioned but shown entire and hiding granule; depression in sheath being formed.

FIG. 51.—Section of cell showing one granule just below pore, and a second down inside chloroplast which is in usual cylindrical form; sheath formation clearly shown.

FIG. 52.—Section of cell just above pore, showing sheath extending through it; one granule concealed by chloroplast while other revealed because a piece of chloroplast is cut away: *h*, hair, *po*, pore; *s*, sheath.

FIG. 53.—Section showing granule suspended in open end of chloroplast: *c*, parts of chloroplast; *cs*, cytoplasmic strands; *h*, hair, *s*, sheath; *ho*, holdfast.

FIG. 54.—Sheath nearly ready to burst.

FIG. 55.—Completed sheath with stream of cytoplasm extending into water.

FIG. 56.—Section showing upper smaller and lower larger granule.

FIG. 57.—Hair twisted into knot.

FIG. 58.—Hair formed in lower cell and extending through pore in upper.

FIG. 59.—Hair extending from adjacent cell into empty cell.

FIG. 60.—Two granules contributing to hair.

FIGS. 71, 72.—Aplanospores, showing much thickened wall and globules.

FIG. 73.—Injured area inside thallus, showing dead cell walls: *w*, rejuvenated cell and not rejuvenated cell after one division; *r*, thickened walls around injured portion.

CERTAIN FOSSIL PLANTS ERRONEOUSLY REFERRED TO CYCADEOIDS

G. R. WIELAND

(WITH FOURTEEN FIGURES)

Just as the fine Colorado cycadeoid found by FERDINAND V. HAYDEN and originally called *Zamiostrobus mirabilis* by LESQUEREUX is now recognized as one of the handsomest pygmic trunks in the silicified group,[1] and just as there are occasional fruits or parts of fruits and even foliage which if better known must fall within the Hemicycadales, so there are on the contrary a few fairly conspicuous and long known fossils which require removal from the cycadeoid synonymy. Among these are especially the *Cycadeoidea abiquidensis* Dawson, which is some early araucaroid cone, or else a *Lepidostrobus* or *Lycostrobus*. Also, there is the singular strobilus or head from the Cretaceous formations of Kansas, originally described by LESQUEREUX as *Williamsonia elocata*, which at least merits a revised description, fortunately made practicable by new material in hand.

Moreover, it appears that the specimens from the North Carolina Trias first referred to *Zamiostrobus* by LESQUEREUX, and then transferred to the cycadeoid stems by WARD, must really be of an araucaroid nature. So far no instance is recalled of confusion between araucarian and cycadeoid cones, although it can be seen that such might arise in somewhat imperfectly preserved specimens; for if in araucarian cones the sporophylls became increasingly sterile, or if in cycadeoid cones the organs called interseminal scales (which are but reduced megasporophylls) became increasingly fertile, a certain similarity of outer form would be reached.

As field exploration is extended, and as the study of plant imprints and casts is made more exact, imperfectly known types reach a larger interest. Too often highly significant fossil plants are so meagerly described or illustrated that they remain virtually un-

[1] American fossil cycads. Vol. II. p. 109. pl. I. Publ. 34, Carnegie Inst. Wash. 1906. 1916.

known to botanists and collectors, and so the search for supplementary or better preserved material that should follow may be delayed indefinitely; or new finds may meet the fate of the initial ones. Surely, as the years pass, hypothetic types of fructification on the borderland between cycads and cycadeoids, and particularly between cycadeoids and the early angiosperms, must be found in increasing number.

Accordingly, in here bringing into view a few types now better understood, along with other forms still uncertain, it is not so much mere points in synonymy that are aimed at; the greater object should be to search out the unusual types because of their suggestiveness in evolutionary study, and keep them assembled. By following that method, types of unusual feature long overlooked or sequestered in minor collections are brought to notice, and unexpected results sometimes appear. Thus while attempting to learn at first hand the characters of some of the little known cycadeoids, a request was made to the custodians of Washburn College, Topeka, Kansas, for permission to examine the isolated type of *Cycadeoidea munita*. Long unseen, this type was supposedly in the collections of that college, but search did not at once reveal it. Instead there was first found and forwarded for study the unique fruit mold described later as *Williamsonia* (?) *hespera*. Another of these debatable types is the so-called *Cycadeoidea abiquidensis* Dawson.

ARAUCARITES (*Cycadeoidea*) EMMONSI (emend.). Fig. 1.—EMMONS, American Geology. p. 123. fig. 92a, 1857 ("trunk of a cycad"); FONTAINE, Older Mesozoic Flora. p. 117. pl. LII, fig. 5 (1883) ("might be called *Zamiostrobus emmonsi*"); WARD, Status of the Mesozoic floras: p. 302 and pl. XLIII, fig. 3. 1900 (*Cycadeoidea emmonsi*); WIELAND, American Fossil cycads. p. 6. 1906 ("trunk of a cycad"); KNOWLTON, Catalogue of Mesozoic and Cenozoic plants of North America. p. 214. 1919 (*Cycadeoidea emmonsi*).

The type here renoted was found by the well known geologist EMMONS in the Triassic (Rhaetic) of North Carolina, over 70 years ago, and is now in the Emmons collection of Williams College. It consists of an ornate, more or less carbonized imprint of small spirally set rhombic scars borne on a thin slab of light colored shale, on the obverse of which is seen an impression of a broad cycadean

leaf. EMMONS called the specimen the "trunk of a cycad"; but later FONTAINE considered it to be a cycadeous strobilus, remarking that it could not be a *Lepidodendron*, because it was "a plant which did not exist in the Mesozoic" (!).

In 1894 WARD first referred this fossil to *Cycadeoidea*, refiguring it, and as just noted, giving an extended description six years later.

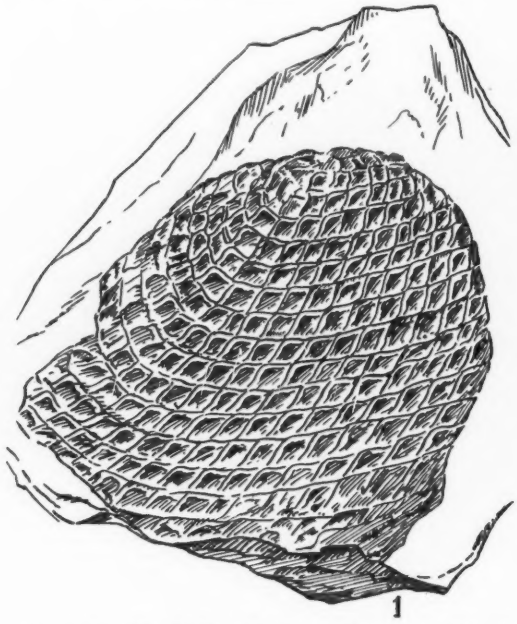


FIG. 1.—*Araucarites* (*Cycadeoidea*, *Zamiostrobus*) *emmonsi* (Emend. Fontaine): cf. with figs. 2, 11, 13; original type from North Carolina Trias (Rhaetic), now in Williams College collection; about natural size.

He is then convinced from an examination of the original specimen that it is the "trunk" of a *Cycadeoidea*, and even suggests some succulent, little woody type, easily crushed. The excellent drawing given is improperly oriented, FONTAINE (1883) having placed as basal what is obviously the apex, although rightly suspecting the fossil to be a cone. It is only 4.5 by 6.5 cm. in size, and might be compared with the English Jurassic cone *Araucarites ooliticus*.

Taking WARD's description and figure, it is certain that this is some kind of araucaroid cone which must be removed from the synonymy of the cycadeoids. He speaks of "ramental walls," but no ramentum was definitely observed, and both FONTAINE's drawing and the better one given herewith show division lines between the scars. Such seldom appear as a persistent limit of the

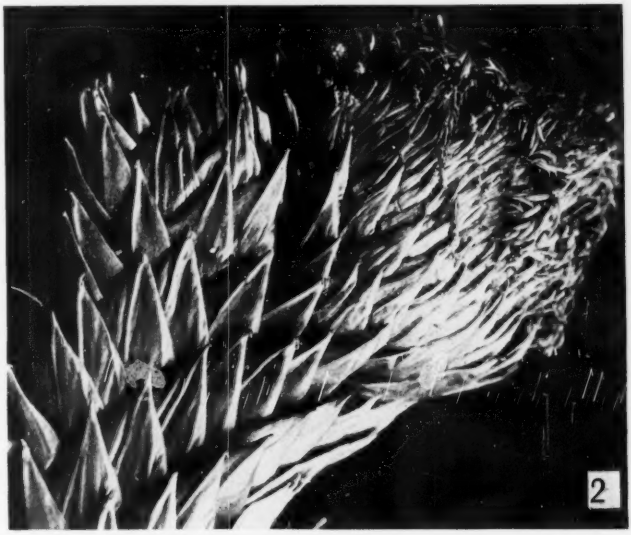


FIG. 2.—*Araucaria imbricata:* one-third to half-grown ovulate cone from Upper Bio Bio Valley, Chile; note on left young vegetative bud, also unwilted straplike ends of megasporophylls, fertile and barren; not oriented; diameter 8 cm.

ramentum borne by adjacent leaf bases. Nor would the ramentum be borne on one side of the bases only. Only in thin sections of petrified material are such lesser features of smaller organs apparent. The lines or "walls" must be merely boundaries between the scales of a cone.

An instance where the scale tips are indicated (compare the present fig. 2) is noted in the clearer araucaroid cone imprint called *Zamiostrobus virginiensis*,[2] which should likewise be transferred to

[2] FONTAINE, pl. XLVII. figs. 4, 5. 1883.

Araucarites. Preservation extending farther out, the scalelike character is more obvious. Also such araucarian cones destined to fossilization would be mostly immature, for at maturity the scales split apart, and fall to the ground to become one of the most frequent of fossil seed and scale types.

FONTAINE himself later says of this fossil (WARD'S Status, p. 301. 1900): "Examined closely with the help of a lens the leaf scars are seen to be of the same character as those of *Cycadeoidea emmonsi*, but decidedly smaller." Doubtless a younger cone!

The name *Zamiostrobus* is an old one given by ENDLICHER, not as satisfactory for this fossil as *Araucarites* Presl, queried if need be. As an araucarian cone, 5–6 cm. in diameter, with the base incomplete, the features are readily reconciled with a quite cosmopolitan series of Mesozoic fossils of similar aspect. Nor is this the first instance of suggested transfer, *Araucarites* having been used by STERNBERG, PRESL, and later authors, "for cones more like those of *Araucaria* than any other conifers."

Furthermore, the huge and more correctly named cone from the North Carolina Trias given in the "Older Mesozoic Flora" on pl. LII, figs. 4, 4a, as *Araucarites carolinensis*, in connection with the illustration of the *Araucarites* (*Cycadeoidea*, *Zamiostrobus!*) *emmonsi*, suggests some partly lepidodendroid features. Such cones could well pertain to the fine stem fragment of pl. XLVIII, fig. 5, of that work.

In bringing *Araucarites emmonsi* and *A. virginiensis* into araucarian association, there yet remains somewhat the same risk as incurred when *Zamiostrobus* was removed to *Cycadeoidea* without first securing some one critical structural detail. Admittedly the time has come when fossil plants cannot be transferred at will from genus to genus on the basis of outer features alone; or worse, the accumulation of debatable points at great length. It is hoped, therefore, that some one at Williams College may attempt a more careful examination of this fossil plant. But it is not this fossil alone which needs study; the series of Rhaetic plants from Virginia and North Carolina should be restudied in entirety, in both the field and laboratory.

At the time coal mining was carried on at Lorraine, Virginia, and

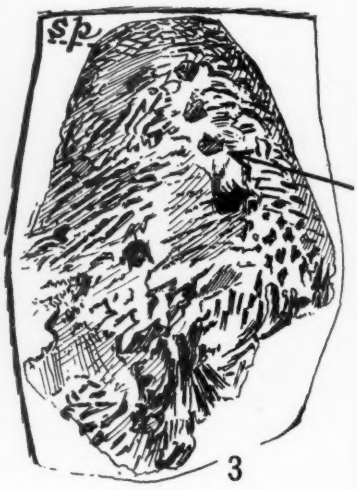
at Egypt, North Carolina, some 40 to 60 years ago, a more remarkable flora was brought to view than could have been obtained by mere exploratory effort. The fossils occur in fine shales, and are nearly always well carbonized, but when they were first studied the only successful closer examination of such plant remains recorded was that of BORNEMANN (1856). Had the chemical methods of imprint study practiced in the past ten years been used on the freshly uncovered Virginia and North Carolina fossils, much more light would have been thrown on this excellent vegetation of the North American Rhaetic, or, as since suggested, Keuper.

ARAUCARITES (?) (*Cycadeoidea*) ABIQUIDENSIS (emend. Dawson). Figs. 3, 4.—DAWSON, J. W., and HARRINGTON, B. A., Geological and mineral resources of Prince Edward Island. p. 45. pl. III, fig. 29. 1871 (*Cycadeoidea abiquidensis*); WARD, L. F., Fossil cycadean trunks of North America. Proc. Biol. Soc., Washington, Vol. IX. p. 87. 1894 (*Cycadeoidea abiquidensis*); WIELAND, G. R., American fossil cycads, Vol. II, p. 109. 1916 (*Cycadeoidea abiquidensis*, of doubtful position).

Professor ADAMS of McGill University some time ago considerately loaned the original Dawson type of "*Cycadeoidea abiquidensis*" for restudy. The Prince Edward Island rocks from which this fossil comes are no longer regarded as Triassic but Permian, from the presence of the Theromorph reptile *Bathygnathus borealis* Leidy (1854), as well as from the geological relations, and in general the plants. While the plant preservation is but fairly good, owing to the prevailing rather granular matrix, considerable carbonization is noted, and further collection afield with study of chemical methods is certainly practical.

The Dawson type comes from the Gallows Point neighborhood of Prince Edward Island, and other examples must doubtless be found. The fossil is certainly not the trunk of any cycad, but a very interesting cone primarily referable to the Araucaroids. As the illustrations show, this cone is preserved in a much flattened carbonized condition. It is about 6.5 cm. long by 4 cm. in diameter, and the photograph (fig. 4) shows the form and features quite as well as they can be shown, unless it should be possible to learn some further facts from successful chemical study. On close scrutiny it

is seen that the organs are set in a pronounced spiral order. Particularly in the area near the summit (fig. 3 *sp*), made from the original Dawson figure, the rhombic pattern of the spirals is un-



FIGS. 3, 4.—*Araucarites* (?) (*Cycadeoidea*) *abiquidensis* (Dawson) from Permian beds of Gallows Point, Prince Edward Island; type in collections of McGill University; slightly enlarged. Fig. 3, drawing made from DAWSON'S original figure of *Cycadeoidea abiquidensis*, so-called, bringing out certain minor and probably accidental markings mentioned in text; arrow denotes these markings, and at *sp* the sporophyll spirals are distinct, as they are over a much larger area than shown. Fig. 4, photograph of original type; arrow shows position of cone axis. This is a crushed though handsome carbonized cone, with sporophyll spirals mostly crushed down. Toward cone apex the rhombic outlines of sporophyll summits and their spiral succession are distinct (length 6.5 cm.).

mistakable. On the specimen itself the spirals can be traced much farther, although in the flattened position.

The position of the base of the central axis is indicated by the arrow in fig. 4. The rhombic ends of the spirals are small, only several millimeters on the faces, and scarcely as large as in a com-

parable Rhaetic cone called by NATHORST *Lycostrobus scotti*. But as SEWARD says (*Fossil plants*), this cone is little different from *Lepidostrobus*.

Preservation would have to be better to decide whether the present cone is araucaroid instead of lepidodendroid. Certainly if Araucarians were derived from, or in any way related to the *Lepidodendron* complex, somewhere about Permian time the transition forms would have borne cones in all outer features very much like those here seen in outline only.

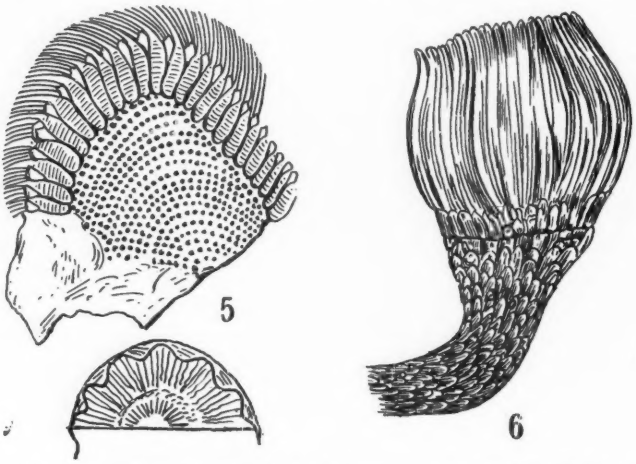
Another feature of the Dawson type is even more uncertain. Scattered about the surface are half a dozen slight bosses, a few millimeters in diameter, indicated in the original drawing and commented on. They scarcely show in the photograph, and are made unduly prominent in the old drawing, as indicated by the arrow. These markings are not branch scars, and certainly are not fruits laterally borne like those of *Cycadeoidea*. They are not in subspiral succession, and cannot be seeds. The best explanation is that they are mere accidental features of preservation in accord with the distinctly granular character of the matrix.

WILLIAMSONIA ELOCATA Lesquereux. Fig. 5.—LESQUEREUX, LEO, Flora of the Dakota group. U.S. Geol. Survey Monograph XVII, pp. 87, 88. pl. II, figs. 9, 9a. 1891; SEWARD, A. C., Fossil plants, Vol. III, p. 462. 1917 (*Williamsonia elongata; elongata* merely a typographical error).

This singular problematic type from Ellsworth County, Kansas, has received no critical notice since its original description. SEWARD gives a bare synonymic mention of it as a fossil of indeterminate feature in his textbook, typographically as *W. elongata*. The original LESQUEREUX type was based on a single specimen, and no further material of interest has turned up until recently, as related in the description of a second form given later, under the caption of a new species. It is desirable accordingly to review LESQUEREUX's description. The *W. elocata* type was submitted by LESQUEREUX to SAPORTA for a critique, and by him referred to the Balanophoreae or "broom rapes." SAPORTA took a variant view of the Williamsonian types as unrelated to cycads, in which he was followed by

several other continental botanists. The type of the *W. elocata* has not been seen for many years. LESQUEREUX says in his description that SAPORTA retained the specimen.

Unfortunately the original drawing of *W. elocata* was reproduced as a halftone; however, the subjoined fig. 5, natural size,



FIGS. 5, 6.—Fig. 5, *Williamsonia elocata* Lesquereux, from Dakota (?) Cretaceous of Ellsworth County, Kansas; head or capitulum above, with imprint or fractured basal surface of such a head below; redrawn from LESQUEREUX; natural size. Fig. 6, *Williamsonia cretacea* Heer, from Atane beds (Atane-kerdluk) of Greenland; length of original 7 cm.; after HEER.

brings out the main features of the original drawing. The description given by SAPORTA as translated by LESQUEREUX follows:

We have recently received from our friend, LEO LESQUEREUX, another fossil organism, or rather the hollow mold of the organism, discovered in the ferruginous sandstone of the Dakota Group, therefore of the Cenomanian. One perceives in the specimen, after molding the cavity in relief, a thick, short receptacle shaped like an ovoidal, conical ball, mostly naked, and marked on its surface by scars of insertion, regularly placed in spiral, of a mass of scales, closely contiguous, inserted at right angles upon the receptacle and surrounded by a thick, spinous apophysis, subulate at base, shorter and less protruding toward the apex of the organism. These scales, which answer evidently to sexual elements, easily disengaged at maturity, are not without analogy, either by themselves or by the structure of the receptacle upon which they were

implanted, with the corresponding parts of the floral spadices of *Williamsonia*. If this analogy is real, we would have here a sessile, naturally caducous receptacle detached after the anthesis from an involucre of which it would have occupied the center. But here, without better evidence, it is difficult to pass above simple conjecture.

LESQUEREUX says of his fruit that it was round or reniform in outline, 4–5 cm. broad, and 3 cm. in vertical diameter; and then he adds an interesting detail unfortunately never illustrated. He states that the fruit was borne upon a cylindrical scaly pedicel or branch 1 cm. in diameter, the scales being 1 cm. long from their enlarged attachment. The cone scales are described as "closely imbricate, flat, heavy behind seeds or bearing pods which are falcate, 1.4 cm. long and 1.5 cm. in diameter, and transversely undulate at the surface as in some small seeds." It may develop, however, that this agrees quite closely with the fossil photographed in figs. 7–10. Faint transverse markings were noted on a seedlike body in one of the sporophylls, but pod cavities, with "small seeds," is an alternative explanation.

Williamsonia (?) **hespera,** sp. nov. Figs. 7–10.—The mold of a gymnosperm strobilus, or mor likely "head" of some dicotyl, 5 cm. in diameter, with the stem which bears it, as shown in the photographic figures, may be a second example of the *W. elocata* of the preceding account. Differences of proportion, however, and perhaps of structure make the use of a new specific name a convenience if not a necessity. In any case this is one of the most curious of ancient fruits. The specimen, as already mentioned, was courteously sent to Yale for study from Washburn College, Kansas, in answer to inquiry concerning the silicified cycad trunk type known as *Cycadeoidea munita*, which has since been found and is a typical cycadeoid of uncommon interest.

No label giving the locality or other fact accompanied the specimen, but several trays full of the characteristic plants from the great dicotyl forests of the Dakota Cretaceous of Kansas, collected 50 years ago by CHARLES STERNBERG and sent to MARSH, afford a clue to horizon and locality.

These leaf specimens are characteristic perfoliate aralia and sassafras-like types; with one or two exceptions readily identifiable

from the figures of dicotyledonous foliage in LESQUEREUX'S monograph of the flora of the Dakota group. The matrix is a soft, often yellowish nodular sandstone, precisely of the same character as that of the Washburn College fossil, so that the latter must be closely associated in some one of the Ellsworth County localities. It is to be added, however, that these Dakota dicotyls extend across several

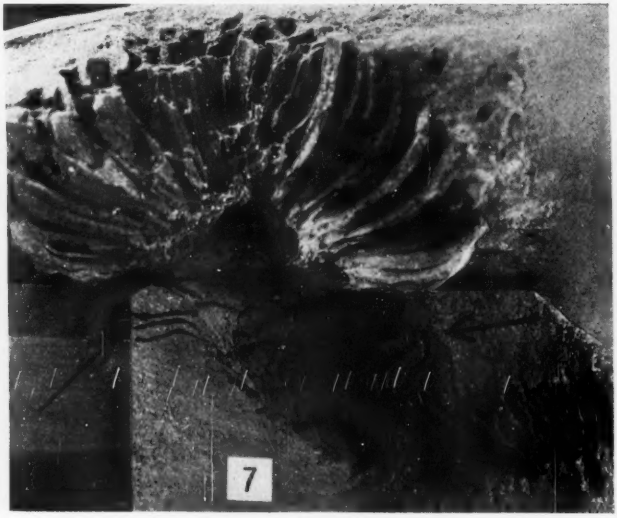
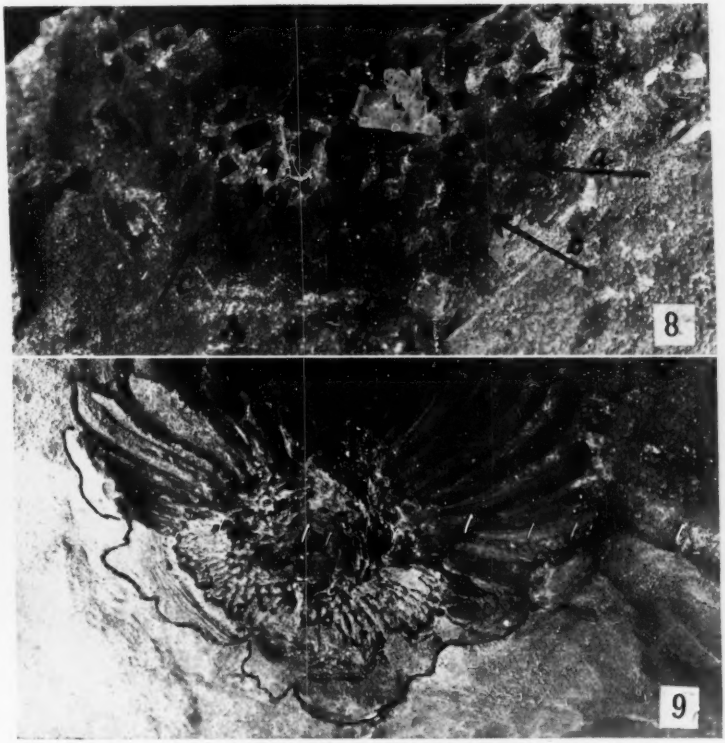


FIG. 7.—*Williamsonia* (?) *hespera*, type from collections of Washburn College; attached stem or peduncle brought into view by saw-cut through empty sandstone mold; cone or head broken open roughly along its diameter, and exactly in place with reference to stem. Note beneath the scale or sporophyll mass the receptacular cavity. Arrows show exact position and length of thin spinose leaves borne by stem or peduncle. Broader scars of bracts just at base of cone or head appear, but not the bracts in this view. (Cone width, 5 cm.; stem length 4 cm.; stem diameter, 8 mm.) See succeeding photographs.

counties, more or less northeasterly. The series in LESQUEREUX'S monograph includes various related forms of leaves from the southwest corner of Cloud County (Glascoe), and the northwest corner of the intervening Scott County (Delphos).

In figs. 7–10 the main features of the new fossil may be visualized. An oblique shearing break through the fructification and across its

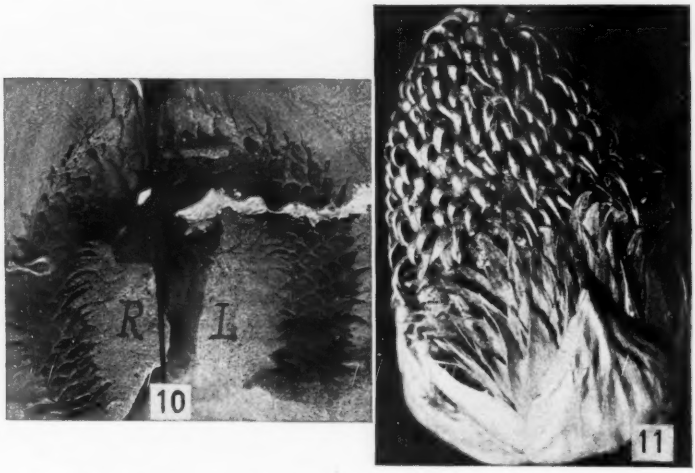
base discloses nearly all details, so far as conserved in the mold. In fig. 8 the fossil is seen from above, and in fig. 9 from below, the



FIGS. 8, 9.—*Williamsonia* (?) *hespera:* Fig. 8, photographed from above; note transversely fractured cavities left by disappearance of scales or carpels, or sporophylls. These transverse fractures in reality are serial from near insertion at center of receptacle (*S*), to broader portion 1.5 cm. out, and thence to bifurcate or paired, spined, or split summits. Note arrows (*a*, *b*, *c*,) pointing out paired spines of scales (if not ends of pods). This fossil is interpreted as a mold formed by imbedding of original capitulum in muddy fine to quicksands. (Diameter of head or cone 5 cm.). Fig. 9, strobilus or head photographed from below, showing broad bract insertions and flatly conical receptacular cavity. This view of base is at level of arrow (*S*) fig. 7. Compare with fig. 5; (diameter 5 cm.).

conical form of the receptacular portion being clearly outlined. Fig. 10 shows the two opposite sides of the attached stem or stalk,

as disclosed by a longitudinal saw-cut through the matrix. It was easy to locate the plane for this cut, as enough of the stem could be seen just below the cavity denoting the receptacular region, to indicate the direction taken by the curved stem through the matrix. The stem ends very abruptly in the matrix, about 4 cm. below the base of the fertile head, as if broken off transversely before fossilization. Even the outlines of the woody cylinder some 3 mm. in diame-



FIGS. 10, 11.—Fig. 10, *Williamsonia* (?) *hespera:* Lower Cretaceous of Kansas. Two opposite halves of cavity or mold of peduncle as brought into view by longitudinal saw-cut; scars of leaf spirals gradually elongate toward bract region; Washburn College specimen. (Stem length 4 cm.). Fig. 11, *Araucaria imbricata:* immature staminate cone from pure stand forests of Upper Bio Bio Valley, Chile; note that this is one of a close-set terminal cluster of five cones, and that following the larger stem leaves are more bractlike leaves basal to cone. Compare with fig. 2; (length 5 cm.).

ter are distinct at the end of the stem mold. Finally, on placing in position one-half of the stem resulting from the saw-cut, the photograph for fig. 7 was made. This comes very near to affording a complete picture, not only of all larger features of the peduncle and head or capitulum, but of much of the structure.

It is first noted that the spirally placed scars of the stem bear rather long leafy scales, or bracts as they must be called, at the strobilar base. These are rather broad and thin, the more distal

portions being somewhat obliterated on the face of the saw-cut. Their precise position and length, as borne along the stem in a close spiral series of nearly imbricant sigmoids, is never in doubt, however, and is indicated by the two arrows (fig. 7). The bracts normally broaden at the transition into sporophylls proper, as can be seen in fig. 9.

FIG. 12.—*Pinus coulteri:* aborted seed cone from San Bernardino Range, California; (diameter 5 cm.).

SPOROPHYLL SERIES

So far, the features might conform to some brachyphylloid or araucarian stem and cone, of Jurassic or Cretaceous age. Photographs showing young ovulate and staminate cones of *Araucaria imbricata* further illustrate the point. Also, the bract features are not inconsistent with the leaves of cone-bearing stems of other conifers, as in an aborted cone of the *Pinus coulteri* from the San Bernardino Range (fig. 12); in fact, the latter figure gives a certain rough approximation to the general size and form of the fossil fruit. It comes near to a restoration, if no attention be given beyond the

fact that the sporophylls (or other structure units) were of about the size and curvature, with diffuse spiral position, seen in the aborted pine cone.

Here, however, analogy ends and doubt begins. Around the bases of the sporophyll areas are small and long lateral slits or cavities, which might have corresponded to about three thin scalelike bodies about each sporophyll or unit. The number is not certain, although if these were infertile scales inclosing one-seeded sporophylls there would be a close approximation to a cycadeoid ovulate fruit. Likewise *Araucaria* would be approached, depending on whatever was the character of the seed; but here another difficulty arises. The sporophyll cavities or units may indeed represent a series of carpophylls, or even podlike fruits, and whatever was their nature they have the curious features of ending in two regularly oriented awl-shaped points about a centimeter in length. The division between these points begins well down in the sporophyll area, and always lies in a plane radial to the center of the cone. Where broken across near their tips *a–c* (fig. 8), the paired sections of these spiny ends are seen to be small and sub-triangular. These paired spines are not an araucarian feature of course, but they do recall somewhat vaguely the two-horned sporophyll of the cycad *Ceratozamia*.

The individual sporophyll areas, as they may be called for convenience, are present therefore as a series of larger cavities in the strictly spiral order. Each is flat to angular, elongate, and bifurcate, instead of "swollen" as in the *W. elocata*. About each larger cavity are the small scalelike cavities denoting unexplained organs. The greatest diameter of the serial cavities, about 3 mm., is reached at a distance of 1.5 cm. out, with bifurcation beyond that point into the two sharp, erect spines, a centimeter or more long. The "sporophylls" or units thus may or may not have been surrounded by, or have included scalelike anthers; and they may have been single seeded or many seeded dehiscent pods.

Relationships

It is difficult as yet to say whether this fruit is a later, highly aberrant form of one of the Cycadeoids, or actually the "head" of

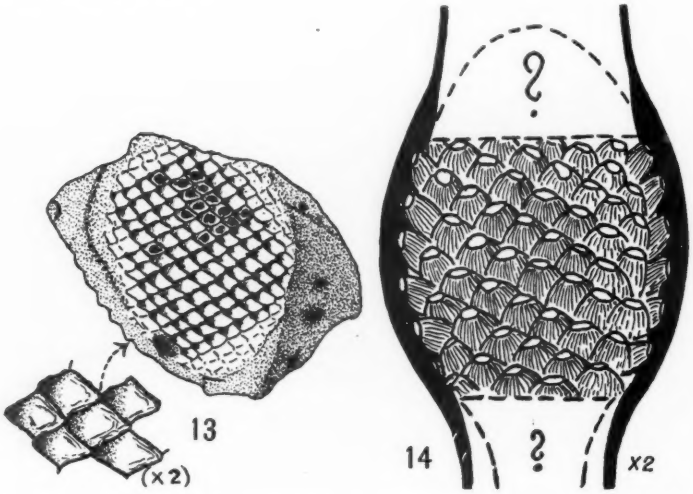
some early dicotyledon. A small body was noted in one instance resembling the cast of a seed about the size of a small grain of rye occupying most of the space, indicating the mold of the "sporophyll." It suggests the presence of a placenta and a series of pods forming a head of some leguminous plant. In a way this fructification is as difficult to understand as *Williamsonia* was originally. That structural riddle solved, this replaces it by one quite as difficult and perhaps as important. A certain relationship to cycadeoid cones depending on the final nature of the sporophylls and surrounding scales is only one of the possibilities. Yet hope of solution does not fail. Flowers found as casts or molds may also occur as impressions, and with both casts and impressions at hand, the anatomical characters might be discerned. The discovery of new material must be awaited.

Generic position

That this type is specifically isolated appears certain, with the exception of the *Williamsonia elocata*. The stem features might agree with some of the various araucaroid or brachyphylloid stems abundant in Mesozoic rocks. But not knowing the family or even the order to which such a fossil may safely be ascribed, and with the hope that other examples may yet be found, there appears no immediate need to apply any other name to it than *Williamsonia*, a name well enough known to signify a whole tribe of extinct plants, as used from time to time.

The curious head, fig. 6, known as *Williamsonia cretacea* of Greenland, should also be recalled here. Heer describes this fossil at some length in the *Flora Fossilis Arctica* (Vol. VI. Mem. II. p. 59), also referring it to the Balanophoreae, as did Saporta and Lesquereux the *W. elocata;* and there seems little doubt that both forms are of the same genus, whatever the family to which they may pertain. It seems, however, to have been Heer and Lesquereux who referred such fossils to *Williamsonia* after Nathorst suggested that the group belonged to the broom rapes, a suggestion he soon emphatically abandoned. Saporta, however, thought the *Williamsonia elocata* should be given a new generic name. Through the fortunate conservation of the greatly specialized silicified cycadeoids, the structure of the cones definitely falling within the genus *William-*

sonia is now understood; but it does not seem that either the Greenland fossil or that just described need as yet be given new generic names in the absence of material eventually to be found. Paleobotanists and well informed botanists know that the fossil genera cannot always be given full precision, and are often highly inclusive, merely genera of convenience. Some fossils are definable within the narrowest limits; others scarcely within the family; the tribe, even the order.



FIGS. 13, 14.—Fig. 13, *Araucarites* (?) *polycarpa* Tenison-Woods from Lower Cretaceous of Urangan Point, Pialba, Queensland, Australia; for comparison with figs. 1, 2, etc. From Walkom; (diameter of cone 3.5+ cm.). Fig. 14, *Bernettia inopinata* Gothan. Rhaetic of Nürnberg: cone of unknown relationship, with Araucarian sculpturing, and large cycadeoid-like outer bract husk. From Gothan; (diameter about 3 cm.).

Conclusion

The fossil plant problems must be kept continually in mind in both the field and the laboratory. Thus alone can progress be achieved in the study of ancient types. Among more recent fossil fruit discoveries of uncommon interest is a bract-enveloped cone from the Rhaetic of Nürnberg, assigned by GOTHAN to the new genus

and species *Bernettia inopinata*.[3] This fossil, fig. 14, is another addition to the forms promising determinate features in the evolution of the great groups. *Bernettia* is outwardly like some reduced araucaroid cone, with the final stem leaves developed into great bracts, recalling *Williamsonia*. GOTHAN places this cone under the Cycadophytes because of the bract features common in *Williamsonia*, with the outer cone features not unlike those of some modern cycad. Yet an araucaroid or even pinoid analogy may quite as well be hypothesized, according with the foliage of Cordaites, or of some one of the many Rhaetic gymnosperms.

GOTHAN also describes from the same region two cycadeoid-like cones, under the new generic names *Piroconites* and *Bennettitacearum*. The former is 5–6 cm. long and pear-shaped, the latter a slightly longer spindle-form fruit. Both have very small sporophylls, and, unlike the foregoing problems, may be assigned to the cycadeoids with some confidence. Even as indistinctly known from surface characters, these cones afford further evidence of abundance and diversity in the cycadeoids or Hemicycadales. Once this great group was known only from the casts and imprints first gathered by WILLIAMSON and his father just 100 years ago; now it is seen to be one of the most remarkable groups of ancient plants. That in the course of fossil plant investigation other Mesozoic groups of high importance in conceptions of the evolution of the flowering plants must come into view cannot be doubted. So many important facts have been learned even now, that the origin of the higher groups of plants is no longer a mystery, as DARWIN said in 1879. Probably botanists, because the fossil plant record is so frequently histologic, ask too severe proofs for origins.

OSBORN BOTANICAL LABORATORY
YALE UNIVERSITY
NEW HAVEN, CONN.

[*Accepted for publication February 20, 1928*]

[3] GOTHAN, W., Die Unter-liassische (Rhaetische) Flora der Umgegend von Nürnberg. Ab. Naturh. Gesell. Nürnberg 4:91–186. pls. 17–39. 1914.

CYTOLOGICAL STUDY OF THE INTRACELLULAR BODY CHARACTERISTIC OF HIPPEASTRUM MOSAIC[1]

FRANCIS O. HOLMES

(WITH PLATE III)

The intracellular bodies characteristic of corn mosaic, sugar cane mosaic, tobacco mosaic, *Hippeastrum* mosaic, and related mosaic diseases have been studied by a number of investigators. Some have suggested that the bodies represent living organisms. The general appearance of the bodies is that of vacuolated cytoplasm, and their close association with the affected areas of the host plants makes the hypothesis seem reasonable. The lack of any visible nucleus in the mass has been the most important argument against the supposition that the body represents some stage in a parasitic organism. In the absence of any substitute for this criterion, the opposing idea that the body represents a waste material or distorted cell constituent has gained a large following.

IWANOWSKI (**7**) in 1903 described the bodies in tobacco mosaic, giving an almost complete description of them as they are known today, but interpreting their granular structure as proof that they were masses of bacteria. KUNKEL (**8**) in 1921 described and pictured the inclusions characteristic of corn mosaic, enumerating the ways in which the bodies resemble amoeboid protozoa, and pointing out the lack of characteristic nuclei. He later published accounts (**9, 10**) of the similar intracellular inclusions in *Hippeastrum* mosaic, sugar cane mosaic, and the mosaic of Chinese cabbage and tobacco. GOLDSTEIN (**2**) and RAWLINS and JOHNSON (**11**) described the appearance of the inclusions in mosaic tobacco. SMITH (**12**) studied a number of infectious and non-infectious chloroses, and found that the vacuolate bodies occurred only in the infectious type. HOGGAN (**6**) recently examined many hosts of tobacco mosaic, and found intracellular bodies consistently in all hosts which showed characteristic macroscopic

[1] Contribution from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

symptoms. She found no such bodies in the same hosts when affected with cucumber mosaic. GOLDSTEIN (3, 4) made extensive studies of the intracellular bodies of tobacco and dahlia, and reported the presence of the bodies in dividing cells, and the division of the bodies there with subsequent distribution by the division of the host cells.

The object of the present paper is to set forth the results of the writer's study of the inclusion bodies which occur in *Hippeastrum* mosaic. The work has been done from the protozoological viewpoint, treating the structure provisionally as though it were a protozoan, the exact systematic position of which was in doubt. It has not been possible to prove whether the structure is or is not a parasitic organism. It has been possible, however, to carry out studies which furnish data not before available. A careful search for nuclei and nuclear material within the body, and an equally careful study of the chondriosome content of the body have been made.

Materials

Almost all of the work herein described was done on a mosaic infected stock of the species *Hippeastrum equestre* (Herb.), which was available at the Institute. Since it proved to be impossible to secure seed from this stock because of clonal sterility, and since no healthy plants were at hand, it was necessary to carry out a part of the investigation on a stock of seedlings obtained from hybrid plants closely resembling this species. The seedlings raised in the greenhouse were invariably free of mosaic, whether from apparently healthy or from the most severely infected parents. They remained completely healthy so long as they were retained in the greenhouse. By placing a portion of the lot of plants in the Institute garden during the summer, with mosaic plants in the same row, enough transmission occurred to give an additional stock of diseased plants.

Hippeastrum was used as a source of mosaic intracellular bodies for this investigation because the bodies in this plant are very large and very numerous, resembling in a general way those of corn and sugar cane.

Search for nucleus in intracellular body

Keeping in mind the hypothesis that the intracellular body might be a parasitic organism, the search for a nucleus or nuclear material

CYTOLOGICAL STUDY OF THE INTRACELLULAR BODY CHARACTERISTIC OF HIPPEASTRUM MOSAIC[1]

FRANCIS O. HOLMES

(WITH PLATE III)

The intracellular bodies characteristic of corn mosaic, sugar cane mosaic, tobacco mosaic, *Hippeastrum* mosaic, and related mosaic diseases have been studied by a number of investigators. Some have suggested that the bodies represent living organisms. The general appearance of the bodies is that of vacuolated cytoplasm, and their close association with the affected areas of the host plants makes the hypothesis seem reasonable. The lack of any visible nucleus in the mass has been the most important argument against the supposition that the body represents some stage in a parasitic organism. In the absence of any substitute for this criterion, the opposing idea that the body represents a waste material or distorted cell constituent has gained a large following.

IWANOWSKI (**7**) in 1903 described the bodies in tobacco mosaic, giving an almost complete description of them as they are known today, but interpreting their granular structure as proof that they were masses of bacteria. KUNKEL (**8**) in 1921 described and pictured the inclusions characteristic of corn mosaic, enumerating the ways in which the bodies resemble amoeboid protozoa, and pointing out the lack of characteristic nuclei. He later published accounts (**9, 10**) of the similar intracellular inclusions in *Hippeastrum* mosaic, sugar cane mosaic, and the mosaic of Chinese cabbage and tobacco. GOLDSTEIN (**2**) and RAWLINS and JOHNSON (**11**) described the appearance of the inclusions in mosaic tobacco. SMITH (**12**) studied a number of infectious and non-infectious chloroses, and found that the vacuolate bodies occurred only in the infectious type. HOGGAN (**6**) recently examined many hosts of tobacco mosaic, and found intracellular bodies consistently in all hosts which showed characteristic macroscopic

[1] Contribution from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

symptoms. She found no such bodies in the same hosts when affected with cucumber mosaic. GOLDSTEIN (3, 4) made extensive studies of the intracellular bodies of tobacco and dahlia, and reported the presence of the bodies in dividing cells, and the division of the bodies there with subsequent distribution by the division of the host cells.

The object of the present paper is to set forth the results of the writer's study of the inclusion bodies which occur in *Hippeastrum* mosaic. The work has been done from the protozoological viewpoint, treating the structure provisionally as though it were a protozoan, the exact systematic position of which was in doubt. It has not been possible to prove whether the structure is or is not a parasitic organism. It has been possible, however, to carry out studies which furnish data not before available. A careful search for nuclei and nuclear material within the body, and an equally careful study of the chondriosome content of the body have been made.

Materials

Almost all of the work herein described was done on a mosaic infected stock of the species *Hippeastrum equestre* (Herb.), which was available at the Institute. Since it proved to be impossible to secure seed from this stock because of clonal sterility, and since no healthy plants were at hand, it was necessary to carry out a part of the investigation on a stock of seedlings obtained from hybrid plants closely resembling this species. The seedlings raised in the greenhouse were invariably free of mosaic, whether from apparently healthy or from the most severely infected parents. They remained completely healthy so long as they were retained in the greenhouse. By placing a portion of the lot of plants in the Institute garden during the summer, with mosaic plants in the same row, enough transmission occurred to give an additional stock of diseased plants.

Hippeastrum was used as a source of mosaic intracellular bodies for this investigation because the bodies in this plant are very large and very numerous, resembling in a general way those of corn and sugar cane.

Search for nucleus in intracellular body

Keeping in mind the hypothesis that the intracellular body might be a parasitic organism, the search for a nucleus or nuclear material

was carried on in every way which seemed likely to lead to success. It was recognized as a general principle that the greatest variety of fixing agents and cytological stains present practically the same picture so long as they act on the same structures; but it was thought worth while to test the typical groups of fixers and stains in the hope that if nuclear materials were present, they might be recognized more easily in the shades of differentiation resulting from the various processes.

The following typical fixing solutions were used: Schaudinn's fluid (a mercuric chloride-fixing agent); Flemming's weak solution (an osmic acid-fixing agent); Bouin's fluid (a formalin picric acid-fixing agent); Carnoy's fluid (an absolute alcohol mixture); and Regaud's fluid (a formalin potassium dichromate-fixing agent). It was felt that these represented the typical fixers needed to insure enough variation in appearance of any structures which might be present in the bodies. The stains used were as follows: iron haematoxylin, destained generally with iron alum; Giemsa's stain, unmodified, staining nuclear material ruby red; acid fuchsin, long stain, destained in tap water; Flemming's triple stain. It will not be necessary in this paper to describe the methods of preparation and use of the fixing agents. For formulas for all of them, and a description of the staining methods, LEE's *Microtomist's Vade Mecum* may be consulted.

In addition to the cytological methods indicated, photography with monochromatic blue light was used to study the general ground material of the body. This light was of wave length 448.1 millimicrons, and was derived from the spark between magnesium electrodes. Many of the blue light photographs, such as are shown in the accompanying plate, show the extremely granular appearance of the bodies. The substance of the intracellular body is frequently very conspicuously granular, even when viewed in unfixed, unstained material. These photographs also show the general contours of the bodies as seen in section. Most of the pictures represent bodies from epidermal cells where no chloroplasts are present. Thus only the cell walls, the cell nuclei, and the inclusion bodies appear. In cells below the epidermis the chloroplasts are often conspicuous, and may be in close contact with the inclusion.

The inclusion bodies react in the same way as the cytoplasm of a young cell does to all types of fixing solutions. This is surprising in view of the fact that they are within the cytoplasm of relatively old host cells. Fixing agents preserve more of their substance than is the case in the nearby fluid cell cytoplasm. Their contours are consequently more easily distinguished in stained preparations than are the contours of the enveloping cytoplasmic strands.

In the search for nuclear material in the inclusion bodies, only two types of structures were found which could possibly be interpreted as nuclei. In most lots of material from mosaic plants the bodies were found to possess a few granules capable of retaining stains intensely. When present, these granules were retained with all the fixing agents used. They stained intensely black with iron haematoxylin, purple with Flemming's triple stain, and brilliant red with acid fuchsin, as do protein granules in many plant cells. They were not observed in material stained with Giemsa's stain, but presumably took a blue stain if they were present, since they were not apparent against the uniform light blue of the intracellular body as stained by this method. Such granules as these are present in the cytoplasm of the *Hippeastrum* plant, and may perhaps represent stored food material. In the absence of any other characteristic than their ability to retain most dyes intensely, they cannot be said to resemble nuclear material very strikingly. Particularly is their absence from material stained with Giemsa's stain an argument against their nuclear nature, as will be mentioned presently. These brightly staining granules have been observed in the intracellular bodies of other mosaic diseases (**8**).

Giemsa's stain is capable of giving a very intense blackish red stain on nuclear chromatin, and a large series of sections of mosaic *Hippeastrum* was therefore prepared with this stain. The stain reacted perfectly in the immediate neighborhood of the intracellular body. Thus the host cell nucleus was so characteristically colored that even the smallest fragment of it was recognizable. The intracellular bodies were immediately adjacent to the well stained plant nuclei, but no nuclear material was shown in them. They stained uniformly light blue, like cytoplasm. It is believed that this Giemsa stain test is evidence that the bodies do not contain nuclear material.

The other nucleus-like structure within the inclusion body is one not previously described. It has the appearance of a sphere or spheroid, possessing one and occasionally two deeply staining balls of material located peripherally. Such spheres may occur singly, or there may be as many as a dozen within a single body. In diameter the spheres range from 0.75 to 2 μ. When present in numbers within a body they are not clumped, but well distributed through the mass. Besides being found in the intracellular bodies, these spheres are sometimes present in the cytoplasm of the host cell. In this location they are clumped in some cases, in others well distributed. They may be scattered between chloroplasts in cells containing such plastids.

Healthy seedlings have never shown such spheres in their cells, although search has been made in more than 50 plants. In plants grown in the field long enough to show slight traces of mosaic infection, these spheres have been found occasionally. In heavily diseased plants the spheres may be present in considerable numbers or entirely absent. Material fixed with Carnoy's fluid, Flemming's weak solution, and Regaud's fluid has been found to retain the structures. It happens that no material fixed with Bouin's fluid or Schaudinn's fluid has ever shown the spheres, but it is entirely possible that this is because too few lots were examined. With most dyes the spheres tend to take a surface stain, but the interior may sometimes be tinted. With iron haematoxylin and eosin the spheres may be outlined in black, or may be uniformly stained with eosin, the peripheral ball being deeply stained with the black of the iron haematoxylin in either case. With acid fuchsin, which stains ordinary nuclei brownish red and nucleoli brilliant red, the spheres are dull red and the peripheral balls brilliant red. With Flemming's triple stain the peripheral balls of intensely staining material become pink, the spheres being outlined in purple. With Giemsa's stain, which stains chromatin ruby red and nucleoli pale blue, the peripheral balls stain deep blue and the spheres stain in outline pale blue.

It is not certain what the nature of these structures is. Since they are the only conspicuous inclusion within the inclusion bodies themselves, an understanding of them may help to solve the ques-

tion of the nature of the intracellular bodies. Somewhat similar structures have been described in the cytoplasm of nerve cells in rabies by GOODPASTURE (**5**), and intranuclearly in human smallpox by CALKINS (**1**).

Chondriosome content of intracellular body

By a study of the chondriosome content of the body it was hoped that light could be thrown on some of the suggestions as to its nature. The numerous hypotheses advanced in the past to explain the intracellular body may be classified into four groups. (1) The body would be expected to have no chondriosomes in its mass if it were an abnormal chloroplast, leucoplast, chromoplast, or elaioplast, normal or abnormal nuclear material, a colony of virus particles free of host cytoplasm, or an abnormal tannin vesicle. (2) It would be expected to contain a moderate number of chondriosomes if it were a parasitic organism, living host cell cytoplasm containing virus, or living host cell cytoplasm without immediate contact with virus. (3) It might be expected to vary in chondriosome content if it were dying host cell cytoplasm or dead cytoplasm. (4) It might be expected to be completely composed of chondriosome-like material if it were a pile of chondriosomes aggregated, or fused, and perhaps chemically changing.

A typical lot of mosaic *Hippeastrum* tissue was fixed in Regaud's fluid, sectioned and stained with a long iron haematoxylin method. The chondriosome content of the cells and of the intracellular bodies was examined in a set of 100 slides.

The preparations consistently pointed to a definite conclusion. The intracellular bodies showed a moderate number of chondriosomes well distributed through their substance, just as the host cell cytoplasm nearby showed its expected quota, also well distributed. A group of photographs of a single intracellular body at different optical levels is shown in fig. 1, to demonstrate the uniformity of distribution of the chondriosomes in the mass of the body. The presence of a moderate number of well distributed chondriosomes in such a mass of material, in itself reacting like cytoplasm to all biological stains applied, is strong evidence for the view that the mass is

partly or wholly living cytoplasm, and is evidence against the opposing hypotheses which have been suggested. Of the possibilities, the three which are not opposed by this finding are: (1) that the body is a stage in the life cycle of a parasitic organism causing the disease; (2) that the body is a mass of host cell cytoplasm containing virus; or (3) that the body is a mass of host cell cytoplasm not immediately associated with virus, although perhaps holding its form because of the stimulation given by the diseased condition.

The hypotheses opposed by this finding of a normal number of well distributed chondriosomes within the intracellular body are also opposed individually by many small bits of evidence. Thus, for example, the probability that the body is not an abnormal elaioplast is indicated further by the fact that normal elaioplasts are found in the stems of mosaic and healthy plants alike, but no intergrading forms occur. Between the three possibilities not opposed by the chondriosome study no choice can be made at present, for no evidence is known to favor any one of them decisively.

Summary

1. A cytological study of the intracellular bodies characteristic of *Hippeastrum* mosaic disclosed the fact that no nuclear material is to be found in the mass of the body, unless two types of structures should be so interpreted. These are (1) intensely staining dots not markedly different from others found outside the body in the fluid cytoplasm of the host cell; and (2) spheres containing deep-staining, peripheral, single or rarely double balls. These spheres are very definitely formed and easy to recognize. They were found also in the host cell cytoplasm in diseased plants, but not in that of healthy plants. These are the only formed structures of distinctive appearance within the intracellular bodies associated with *Hippeastrum* mosaic. It has not been possible to identify them.

2. Chondriosomes were found within the intracellular bodies in moderate numbers, well distributed through the mass. This observation is considered evidence for the view that the intracellular body in this particular disease consists of living cytoplasm. Whether the body represents a stage in a foreign organism, a mass of plant cell

cytoplasm containing virus, or a mass of the plant cell cytoplasm not immediately in contact with virus but stimulated by the diseased condition, is not known.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.
YONKERS, N. Y.

[*Accepted for publication March 24, 1928*]

LITERATURE CITED

1. CALKINS, G. N., *Cytoryctes variolae* Guarnieri. Jour. Medical Research 11:136. 1904.
2. GOLDSTEIN, B., Cytological study of living cells of tobacco plants affected with mosaic disease. Bull. Torr. Bot. Club 51:261–273. 1924.
3. ———, A cytological study of the leaves and growing points of healthy and mosaic diseased tobacco plants. Bull. Torr. Bot. Club 53:499–599. 1926.
4. ———, The x-bodies in the cells of dahlia plants affected with mosaic disease and dwarf. Bull. Torr. Bot. Club 54:285–293. 1927.
5. GOODPASTURE, E. W., A study of rabies, with reference to a neural transmission of the virus in rabbits, and the structure and significance of negri bodies. Amer. Jour. Pathology 1:547–582. 1925.
6. HOGGAN, I. A., Cytological studies on virus diseases of solanaceous plants. Jour. Agric. Research 35:651–671. 1927.
7. IWANOWSKI, D., Über die Mosaikkrankheit der Tabakspflanze. Zeitschr. Pflanzenkrank. 13:1–41. 1903.
8. KUNKEL, L. O., A possible causative agent for the mosaic disease of corn. Hawaiian Sugar Planters' Assn. Bull., Bot. Series 3:44–58. 1921.
9. ———, Amoeboid bodies associated with *Hippeastrum* mosaic. Science 55: 73. 1922.
10. ———, Further studies on the intracellular bodies associated with certain mosaic diseases. Hawaiian Sugar Planters' Assn., Bot. Series 3:108–114 1924.
11. RAWLINS, T. E., and JOHNSON, J., Cytological studies of the mosaic disease of tobacco. Amer. Jour. Bot. 12:19–32. 1925.
12. SMITH, F. F., Some cytological and physiological studies of mosaic diseases and leaf variegations. Ann. Mo. Bot. Gard. 13:425–484. 1926.

EXPLANATION OF PLATE III

All photographs were taken with blue light from the magnesium arc, wave length 448.1 millimicrons.

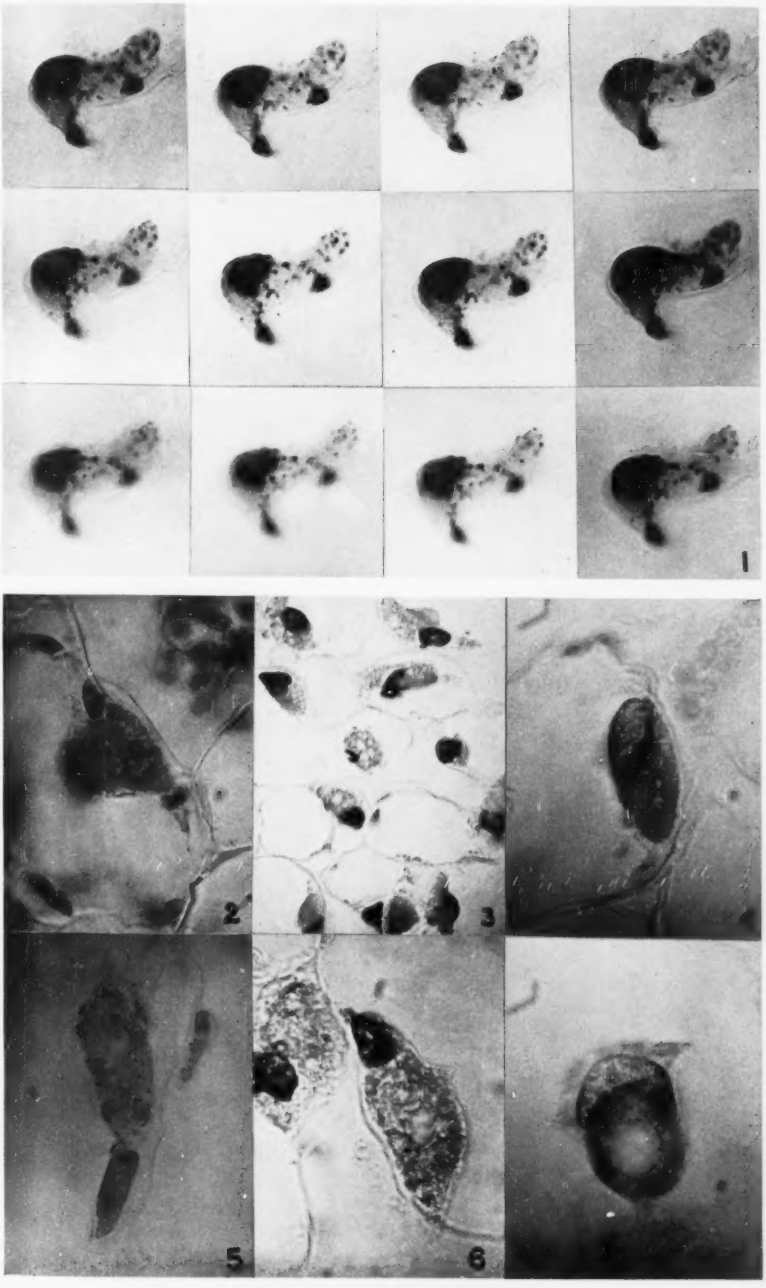
FIG. 1.—Nucleus and adjacent intracellular body photographed at twelve different optical levels to show that the dotlike chondriosomes are distributed

through mass of body and not merely on its surface; from mosaic *Hippeastrum* tissue fixed in Regaud's fluid and stained with iron haematoxylin; nucleus at left, intracellular body containing well distributed chondriosomes at right; two plastids below nucleus and body against wall of cell; ×1000.

FIG. 2.—Intracellular body containing five spheres, only one showing in photograph; peripherally located deeply staining dot can be distinguished in sphere-like structure; ×1000.

FIG. 3.—Low power photograph of section through epidermis of mosaic *Hippeastrum* plant; nuclei of cells appear very dark, bodies vacuolated and granular; fixed in Schaudinn's fluid, stained with Giemsa's stain; ×300.

FIGS. 4–7.—Intracellular bodies showing granular structure, especially in fig. 6, vacuoles in figs. 6 and 7, close contact with nucleus in all; figs. 4, 5, and 7 from material stained with acid fuchsin following Bouin fixation; fig. 6 from material fixed in Schaudinn's fluid and stained with Giemsa's stain; ×1000.



HOLMES on HIPPEASTRUM

ULTRA-VIOLET LIGHT PHOTOGRAPHY IN THE STUDY OF PLANT VIRUSES[1]

FRANCIS O. HOLMES

(WITH PLATE IV AND ONE FIGURE)

It has seemed desirable for some time that photography with ultra-violet light, using a quartz-lens microscope, should be applied to the study of the infective juices of the virus diseases of plants. It was realized before the present work was undertaken that there were many difficulties which might make it impossible to obtain successful results. There existed the chance, however, that some one of the viruses available might consist of formed particles just too small to be seen with the visual microscope. If these particles were not too small and should possess a favorable refractive index, their images might appear on photographic plates exposed with light of short-wave length. The careful examination of these plates at leisure might make it possible to recognize structures characteristic of the virus-containing samples.

In order that this method of observation of the plants infected with a number of viruses might be thoroughly tested, arrangements were made with the Botany Department of Columbia University, through the kindness of Dr. R. A. HARPER, whereby their Zeiss ultra-violet light photomicrographic apparatus was made available to the Boyce Thompson Institute for Plant Research for a period of two years. It was hoped that a fairly complete preliminary survey of this field of investigation could be made in that length of time. The purpose of this paper is to report the methods used, the types of viruses studied by this means, and the results.

Ultra-violet light photomicrographic apparatus

The apparatus used in the pursuit of these studies is shown in fig. 1. It was devised by KÖHLER, who described it thoroughly in

[1] Contribution from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

a long paper.[2] STEMPELL[3] used this apparatus in his research which resulted in the photographing of the spiral filaments of *Nosema bombycis* within the spore. The apparatus is constructed to allow the sorting out of light of low-wave length from the cadmium spark, and the isolation of a relatively pure beam of light corresponding to the wave length of 275 millimicrons. This light is led through a

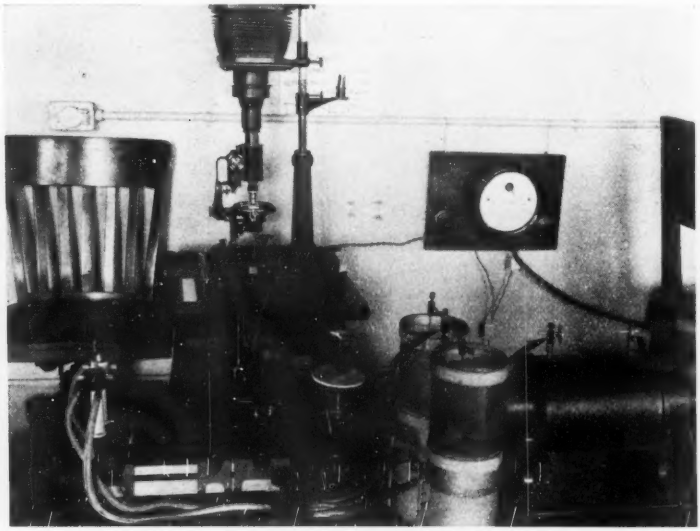


FIG. 1.—Zeiss apparatus for ultra-violet light photography: light obtained from a spark between cadmium or magnesium electrodes; quartz lenses and prisms isolate light of desired wave lengths and conduct it to microscope, which is equipped with quartz lenses.

series of quartz prisms, and passed through the microscope, which is provided with excellent quartz lenses adjusted for this exact wave length. Since the light is monochromatic, no chromatic aberration interferes with the sharpness of images secured. Objects to be photographed must be unstained and mounted between quartz slides and cover slips to insure complete transmission of the ultra-violet light.

[2] KÖHLER, A., Mikrophotographische Untersuchungen mit ultravioletten Licht. Zeitsch. Wissen. Mikr. **21**:148, 273. 1904.

[3] STEMPELL, W., *Nosema bombycis*. Archiv für Protistenkunde **16**:281–358. 1909.

The virus-containing juices used were quite satisfactory, in that they contained just enough débris to make it certain that focusing on the several layers of a given mount was accurate, and that suitable small objects within the range of the instrument were being depicted.

Typical viruses examined

The viruses to be studied were chosen with care to represent as varied types as possible. They were the following: aster yellows, tobacco mosaic, tobacco ring spot, potato witches' broom, potato leaf roll, potato rugose mosaic, potato aucuba mosaic. The aster yellows material was available at the Institute, where insect transmission experiments were under way. This virus causes chlorosis and the development of secondary buds, but no mottling or necrosis on the leaves. It is not mechanically transmissible. In complete contrast to this disease were tobacco mosaic and tobacco ring spot. The mosaic causes intense mottling with profound distortion of leaves, but with no necrosis; ring spot causes a necrotic pattern of concentric rings, but produces little chlorosis. Both of these tobacco viruses are easily transmitted mechanically, in contrast to the aster yellows virus which resists all attempts at transfer by rubbing or related methods.

On potato, additional types were made available through the kindness of E. S. SCHULTZ of the U.S. Department of Agriculture. The first of these was witches' broom, which is not related to aster yellows, but which causes growth of buds normally remaining dormant, thus simulating the appearance of aster yellows plants with less yellowing of the leaves. The second virus from potato was leaf roll, differing from all those thus far mentioned in causing no strong chlorotic or necrotic pattern on the leaf surfaces, but distinguished by a characteristic roll of the leaves. The third of this group was potato rugose mosaic, a mosaic comparable with the strong mosaic of tobacco in that it is readily transmitted mechanically. The fourth and last of the potato juices photographed was that containing the virus of aucuba mosaic, which causes isolated circular chlorotic spots on the foliage, but causes no noticeable leaf distortion or crinkling.

To collect a more varied group of viruses than these would be

difficult. It was necessary to have as representative a group as possible without introducing too many individual viruses, because the labor involved in properly studying a single additional type is considerable. It was hoped that these viruses might differ among themselves fundamentally, and that if size of the infective agent was the factor which has made it impossible to discover what viruses really are, some one of the seven might prove favorable in this respect, and furnish structures within the size range of the ultra-violet light instrument.

The juices to be photographed were obtained from the petioles of leaves of affected plants by pressing these against the quartz slide to secure a drop of fluid together with some débris from the broken cells. Such fluids in the case of tobacco mosaic are capable of causing the disease when introduced into healthy plants. The fluid was mounted for photographing between a quartz slide and a small quartz cover slip, and sealed in place with a ring of vaseline. The whole mount was placed at once under the microscope. A rough focus was secured with daylight by placing in the field of vision an air bubble purposely admitted into the preparation. This air bubble was still visible when ultra-violet light was passed through the preparation and caught on the fluorescent finder provided for the purpose. It could then be moved by means of the mechanical stage to a position at the edge of the field where it would appear in pictures taken at low magnifications, but not in the restricted fields taken by the more powerful quartz oculars.

Following the more careful adjustment of the focus by means of the fluorescent finder, a series of strip pictures was taken with a low power quartz ocular, the fine adjustment setting being recorded at each exposure. The plate thus secured was developed at once. With the best focus established, work began in earnest. The low power ocular was replaced by a higher. No inaccuracy was introduced in this way, as the lenses were made well enough to be in absolute agreement in giving a sharp focus. The same quartz objective was used throughout. A series of pictures was then taken, two on each plate, beginning with a point just below the correct focus and continuing to a point just above this focus. This allowed

a series of pictures to be examined for each spot of virus-containing juice photographed. It was found desirable to have at least a little débris from the plant tissues in each field to make possible an accurate comparison of the different levels, and to give assurance that delineation was satisfactory under the working conditions from day to day.

Difficulties were met in keeping the light intensity uniformly high, since a very slight change of direction of the beam causes a decrease in the illumination of part of the field at least. With light from the cadmium spark passing obliquely through a small eccentric stop and the highest objective, no decrease in intensity is permissible. Vibrations were eliminated as far as possible. The sealing of the plant juice in the mount and its subsequent exposure to ultra-violet light might seem to endanger the virus, yet motile bacteria handled similarly did not seem to be harmed, preparations after photographing appearing as fresh and as active as before.

It may be well to consider the conditions under which ultra-violet light photographs would be unable to depict small objects. If all the units to be studied were in active motion, whether showing Brownian movement or independent activity, no satisfactory images could be obtained. Among the most actively swarming bacteria, however, there are always some at rest on the surface of the cover slip, and the same is true of particles most of which may be in Brownian movement. If the refractive index were like that of the surrounding medium, and the absorption of ultra-violet light were low, particles would fail to impress their images on the photographic plate. The absorption of light by very small objects is in fact quite low. Small bacteria do not hold back much light, but because of their refractive indices they make distinct images on the photographic plate. The refractive index is sufficiently high in all known organisms to allow of photographing, but it might conceivably be low in the viruses. If this should be the case it might be impossible to see or photograph them. If, again, the causative agent were an organized structure smaller than 70 millimicrons in diameter, it would be hopeless to attempt to depict it with the type of short wave length apparatus now available. It would then be necessary

to have a powerful source of light of shorter wave length than 275 millimicrons to succeed.

In this investigation the writer was concerned only with the range between the limit of resolution of the visual microscope, with particles in the neighborhood of 150 millimicrons in diameter, and the limit of the ultra-violet light instrument with oblique light with particles approximately 75–80 millimicrons in diameter. The results obtained from the whole study were negative. In the virus-containing juices of the seven diseases investigated, no formed particles were observed to differ from those depicted in the juices from corresponding healthy plants. A careful search was made through the extensive series of negatives obtained during two winters' work, but no structures characteristic of the infective juices could be detected.

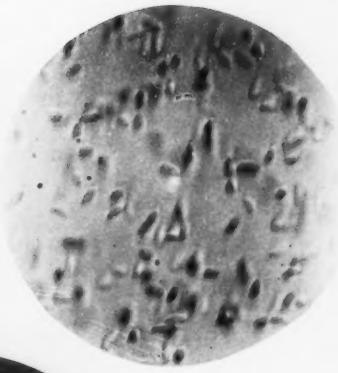
About 600 photographs were made of the virus-containing juices. In addition a series of photographs of known bacterial plant pathogens was made for comparison. This series included *Bacterium tumefaciens*, *Bacillus amylovorus*, *B. caratovorus*, *Bact. campestre*, and *B. melonis*. The species were all photographed without staining, using living organisms. The results show that the ultra-violet light photomicrographic apparatus is of value in the study of the precise morphology of living, unstained bacterial organisms. It is already well known to be suited to the study of minute fungi and algae. KÖHLER and KRUIS[4] have published photographs of a number of species of living bacteria photographed in this way with ultra-violet light without the use of stains, but as the species were mostly unfamiliar, and KRUIS's publication is very difficult to secure, the five species of plant pathogens just mentioned are shown in an accompanying plate.

Summary

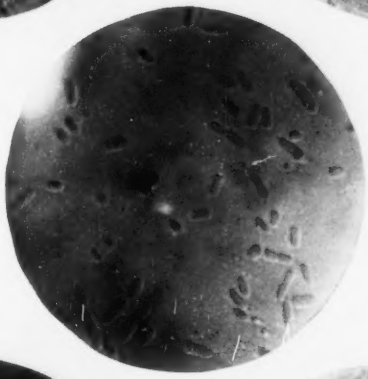
1. Seven typical juices from plants affected with virus diseases were photographed with ultra-violet light of wave length 275 millimicrons, but no formed structures other than those seen in corresponding fluids from healthy plants were found.

[4] KRUIS, K., Rozpravy ceské akademie císaře Františka Josefa pro vedy, solvesnost a umění. Třída 2, R. 22:23. 1913. (Bacteria photographed with ultra-violet light.)

1



2



3

4

5

HOLMES on PLANT VIRUSES

2. The plant juices photographed were from asters having aster yellows, tobacco infected with tobacco mosaic and tobacco ring spot, potatoes having witches' broom, leaf roll, rugose mosaic, and aucuba mosaic.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.
YONKERS, N. Y.

[*Accepted for publication March 24, 1928*]

EXPLANATION OF PLATE IV

All the figures are of unstained living bacteria, ×2000.

FIG. 1.—Bacillus caratovorus.

FIG. 2.—Bacillus amylovorus.

FIG. 3.—Bacillus melonis.

FIG. 4.—Bacterium tumefaciens.

FIG. 5.—Bacterium campestre.

ACCURACY IN QUANTITATIVE WORK WITH TOBACCO MOSAIC VIRUS[1]

FRANCIS O. HOLMES

(WITH THREE FIGURES)

It is often desirable, in working with the readily transferred virus of tobacco mosaic, to have some means of telling whether one sample of the infective juice is more infectious or less so than another. This has frequently been accomplished by inoculating from ten to fifty plants in one of the accepted ways of mechanically transmitting the disease, and then judging from the number of resulting infections which of the two sources contained the larger proportion of virus in a given volume. The accuracy of this procedure is sufficient to allow a correct differentiation to be made between strong and weak samples of virus. Samples differing but slightly in strength can be differentiated and properly graded only by inoculating larger numbers of plants.

A recent paper by MCKINNEY[2] recommended a standard procedure for quantitative studies. He emphasized the difficulty of growing large enough numbers of plants in the greenhouse space usually available. The methods described in the present paper allow quantitative work to be done with economy of space, convenience, and rapidity of manipulation while inoculating, and a high degree of accuracy. It is particularly important to know what accuracy may be expected in experiments with viruses of known relative concentrations. With this information in hand the numbers of plants needed for proposed studies may be calculated.

During the study of plant disease viruses by means of ultraviolet light photography, it was desired to know how many units of virus, if such exist, were to be found in the small volumes of

[1] Contribution from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

[2] MCKINNEY, H. H., Quantitative and purification methods in virus studies. Jour. Agric. Research **35**: 13–38. 1927.

material represented in the individual photographs. For this reason the methods described in this paper were worked out. It was later found possible to obtain data from which the accuracy of such quantitative determinations of virus strength could be calculated, and from which the numbers of plants required to perform given inoculation experiments could be estimated in advance.

Procedure in making measurements

Since large numbers of inoculations were to be made, in order to reduce the effects of chance errors, it was evident that some uniform but very rapid method of performing the inoculations would

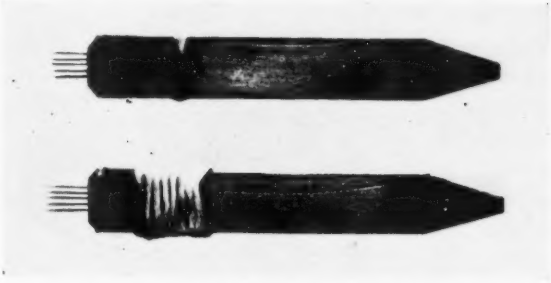


FIG. 1.—Inoculating needles, described in text

be needed. Punctures made with black enamel insect pins, size no. oo, were found to produce wounds of exceedingly regular size. Through these pin pricks tobacco mosaic was readily transferred, merely by alternate punctures in leaves containing virus and in leaves of healthy test plants. The pins used were obtained fresh for each experiment, so that no contaminations occurred because of the inoculating instruments. The fine points of the pins penetrate the leaves with the slightest pressure. It is not necessary to touch the plants at any time with fingers or instruments other than the insect pins actually used to introduce the inoculum. The uniformity of the dose lifted on the pins and transferred to new plants from mosaic specimens or from extracted juice is evident from the results. To obtain a fair percentage of infections it is necessary to make about five pin pricks in each test plant. The work of transfer

is therefore very greatly facilitated by using, instead of a single insect pin, a set of five pins bound in a temporary handle between two wooden pot labels (fig. 1). Rapidity of inoculation and uniformity of dose being thus combined, it is possible to make the required inoculations at the rate of 500 or more an hour. This method was found to be very satisfactory, and allowed many interesting experiments to be performed. Greenhouse space must be conserved if large numbers of determinations are to be made. The use of the simple inoculating apparatus just described makes it possible to utilize small tobacco plants. The wounds made by the pin prick inoculations into the leaf tissue are so small and heal so readily that even young plants are not held back by the introduction of the inoculum. No dead tissue appears around the site of the inoculation, as happens sometimes with rougher methods. It has been suggested that such dead tissue may hold back part of the virus applied and prevent its entry, so that the clean healing under the circumstances seems favorable.

In all of the experiments described in this paper, Turkish tobacco was used, because of the desirable shape of the plant for greenhouse studies, and because of its marked susceptibility to the mosaic disease. Its upright habit of growth and short leaves economize space. The tobacco plants were transferred to flats when they had a leaf spread of about one inch, and when well established were used for inoculation. The results can often be obtained before serious crowding begins, but crowding never caused trouble by the accidental transfer of the disease. Judicious care in handling plants and freedom from insects allow tobacco mosaic experiments to proceed with practically no contaminations.

In fig. 2 are shown the types of plant used. The wooden flat at the left contains small plants which have just been transplanted to it. The middle flat shows plants established and ready for inoculation. The flat at the right contains plants already diseased and ready to be discarded. So many plants can be placed in a greenhouse bench, when they are thus planted in wooden flats, that the space requirement for performing extensive experiments in a quantitative way is reduced to a minimum.

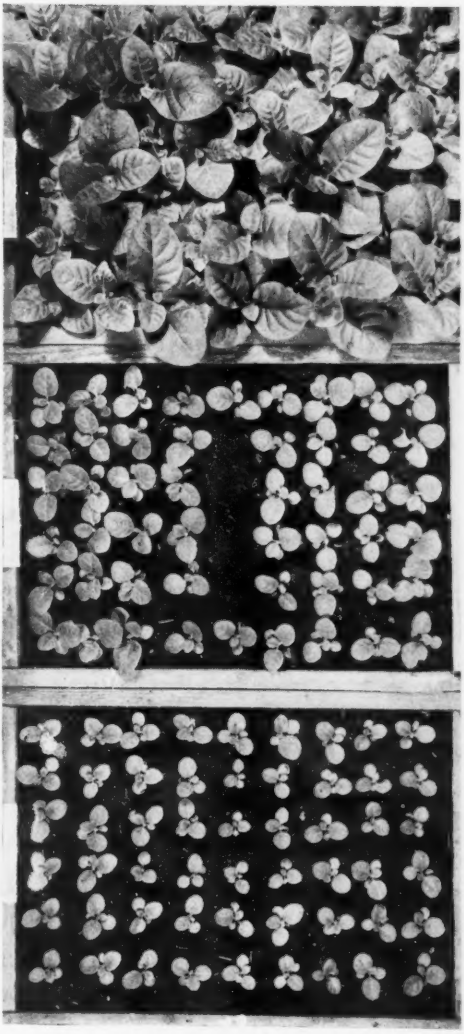


FIG. 2.—Turkish tobacco plants in wooden flats as used for quantitative measurements of tobacco mosaic virus strengths: at left, plants just transferred to flat; center, plants a week later when well established and ready for inoculation; at right, plants after results of inoculation are recorded, flat ready to be discarded.

Results in measuring known samples

In order to know the errors involved in measuring the strengths of virus samples, experiments with definite strengths of virus were needed. A stock virus was therefore made by cutting 100 or more mosaic plants of Turkish tobacco into small pieces and expressing their juices into a glass bottle. The actual strength of this stock virus at any one moment was not known, but by measuring out one portion of this virus and mixing with it three portions of water, it is clear that a new sample of the virus is obtained, the total virus content of which, in a given volume, is one-fourth that of the original

TABLE I*

REDUCTION IN NUMBER OF INFECTIONS IN EACH SET OF 50 TEST PLANTS THROUGH USE OF KNOWN DILUTION

COMPARISON OF UNDILUTED VIRUS WITH	MEAN REDUCTION IN EACH SET OF 50 TEST PLANTS	P.E. SING.	NO. OF SETS COMPARED WITH UNDILUTED VIRUS	P.E. MEAN	STANDARD DEVIATION
1:2.....	3.7	4.5	20	1.0	6.5
1:4	7.5	4.6	24	0.9	6.7
1:8.....	11.2	3.3	16	0.8	4.8
1:16.....	15.3	5.2	16	1.3	7.4
1:64.....	22.4	2.2	8	0.8	3.1

* For compactness in this table the mean reductions are given. As an example of the original figures obtained in the experiments, there follow the paired observations on undiluted and diluted samples from which the values shown in the 1:8 item above were calculated: 35–29, 29–8, 36–28, 28–12, 22–11, 11–4, 20–11, 11–3, 42–29, 29–19, 44–27, 27–21, 44–27, 27–20, 34–28, 28–11. It will be noted that the reduction on dilution is independent of the strength of the undiluted sample, as is implied by the graph shown for these values in fig. 3.

sample. As will be shown later, this new sample upon inoculation into test plants does not give a reading one-fourth that given by the original sample, although it contains only one-fourth as much virus per unit volume. The effectiveness of a dilution for purposes of inoculation is not reduced by the addition of water so rapidly as is the actual concentration.

By adding water to the stock virus in other proportions, other definite dilutions may be obtained. A considerable number of such dilutions were prepared and all were promptly used for inoculation. Fifty inoculations require exactly five minutes. No great deterioration would be expected in such short intervals, and the consistency of the readings indicates that deterioration did not interfere with the process. In measuring known dilutions 2500 plants were used.

When the results were tabulated, as shown in table I, it was found that dilution to one-half original strength causes on the average a drop of 3.7 ± 1.0 infections per set of 50 plants inoculated. This drop is independent of the strength of the original sample over the range studied. A dilution to one-fourth results in a decrease to the extent of 7.5 ± 0.9; a dilution to one-eighth causes a decrease of 11.2 ± 0.8; the still greater dilution to one-sixteenth results in lowering the reading by 15.3 ± 1.3; and finally the dilution to one-sixty-fourth of the original strength causes a decrease in the number of infections amounting to 22.4 ± 0.8 plants. A graph from which intermediate points may be read is shown in fig. 3.

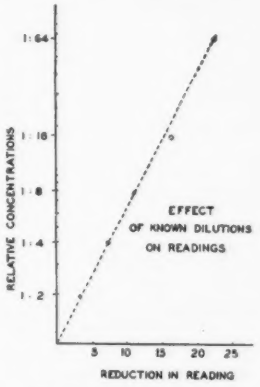


FIG. 3.—Graph showing effect of reducing virus strength by dilution; reduction in reading due to dilution is expressed in terms of average difference in number of infections resulting when undiluted and diluted samples are used, each in a set of 50 test plants.

As will be noted from the graph, the character of this response to dilution is such that the original strength of the virus does not affect the numerical reduction in infections resulting from the dilution. The numerical reduction due to dilution is dependent upon the degree of dilution, but not upon the strength of the sample taken for dilution. This makes it possible to use the information contained in the graph without the necessity for obtaining a virus of standard strength. With this information in hand, it is possible to calculate at any time the number of plants required to perform a given experiment in which it may be desired to differentiate between two viruses of moderately different strengths, or in which it may be necessary to compare two viruses with accuracy. If no limit to the number of plants available existed, it would be possible to obtain any desired degree of accuracy. Future improvements in technique may make it possible to determine virus concentrations as accurately as bacterial numbers may be estimated or chemical concentrations may be calculated from quantitative analyses.

For the present it seems necessary to be satisfied with a degree of accuracy demanding only a few hundred seedling plants. Table II shows the number of plants required to determine with good odds for significance the order of concentration of samples of virus of various strengths. Thus to distinguish with fair certainty between an undiluted sample and one a sixty-fourth as strong, some 30 plants are needed for each of the two samples in the comparison. If the difference between the two samples is but one to sixteen, 60 plants are required. If the difference is one to eight, 120 plants will suffice. If one to four, 240 plants must be used. If one to two, 1350 plants

TABLE II

NUMBERS OF PLANTS REQUIRED TO DETECT MODERATE DILUTIONS

$N = \left[\frac{\text{C.O.} \times \text{P.E. sing.}}{\text{Allowable deviation}}\right]^2$ Average of P.E. sing. of difference between two dilutions = 4.0 (see table I)*

C.O. for odds of 100:1 = 3.9 C.O. × P.E. sing. = 3.9 × 4.0 = 15.6

To compare undiluted virus with	Numbers required for each dilution; to be compared with same number from undiluted sample
1:2	$(15.6)^2 \div (3)^2 = 27$ sets or 1350 plants
1:4	$(15.6)^2 \div (7)^2 = 4.8$ sets or 240 plants
1:8	$(15.6)^2 \div (10)^2 = 2.4$ sets or 120 plants
1:16	$(15.6)^2 \div (14)^2 = 1.2$ sets or 60 plants
1:64	$(15.6)^2 \div (20)^2 = 0.6$ sets or 30 plants

* The probable errors of single observations as listed in table I are so nearly equal, and have in themselves so large a probable error, that in calculating the numbers of plants required for experiments their average is used.

will be essential for the high degree of certainty implied by the odds of 100:1 that the difference observed is significant. It seems worth while to use these large numbers of plants since the labor involved is not great. The greenhouse space demanded is moderate compared with that often used in inaccurately determining virus strengths on smaller numbers of large plants. The equipment needed for the experiments is simple, being new insect pins for each experiment and a few wooden pot labels.

The formula by means of which table II was constructed may be of interest to some, since it is necessary in determining for any new experiment the numbers of test plants to be used. It is: $N = \left[\frac{\text{C.O.} \times \text{P.E. sing.}}{\text{Limiting deviation}}\right]^2$. In this formula N stands for the number

of plants to be used in the experiment contemplated; C.O. signifies the coefficient of odds corresponding to the degree of certainty desired (see PEARL and MINER[3] for complete table; 3.9 may be inserted here in the formula if odds of 100:1 for a significant result will be satisfactory); P.E. sing. stands for the probable error of a single comparison; limiting deviation is to be replaced in the formula by the figure representing the allowable error beyond which the set of readings must not go in the experiment to be performed, lest the significance of the results be impaired.

Some applications of the method

EFFECT OF STORAGE ON SAMPLES OF PLANT JUICE CONTAINING VIRUS

It is evident that, since the error of experiments can be known accurately and adjusted within desired limits, many interesting questions can be answered, using enough plants in each case to obtain trustworthy results. Viruses from different sources may be graded accurately according to their initial concentrations, methods of preserving viruses may be compared as to their efficiency, and treatments with chemicals may be interpreted in the light of their exact effects. One very interesting and practical problem concerns ways of storing virus so as to have as slow a change in strength as possible. The practical importance of the matter comes from the need for some method of holding a given virus from season to season in order that experiments made at different times may be comparable. For example, it is desirable to know whether susceptibility of host plants differs with the change from summer to winter growing conditions, as some have suspected. This problem cannot fully be solved until some fixed reference point, such as a stable stock of virus, has been secured. It has long been known that tobacco mosaic virus allowed to stand in a bottle in the laboratory remains infectious for years. The question has never been answered, however, as to how rapidly the virus may lose strength at first. Most observers have noted diminished potency after long standing.

A rather extensive experiment was performed as follows. A

[3] PEARL, R., and MINER, J. R., A table for estimating the probable significance of statistical constants. Maine Agric. Exp. Sta. Bull. 226. 1914.

quantity of fresh virus, amounting to some 300 cc., was divided into two lots. One-half was stored in a glass bottle on the laboratory desk at approximately 22° C.; the other half was stored in a room kept below the freezing point. Before the latter sample was frozen it was poured into tubes. One-half of these tubes were allowed to stand frozen, the other half were melted and refrozen ten times. Each time the virus was poured from tube to tube in such a way that the clear ice of high melting point was gradually separated from the brown mother liquor of low melting point, and this in turn from the green sediment which contained all the solid débris.

Tests with virus kept at room temperature

The first test of the fresh virus was made at once upon the collection of the juice. Five flats, each of 50 plants, were used for a series of small dilutions of this juice, the same dilution series being used throughout the course of this whole experiment, so that the total readings from the series could be compared each time with other totals. As soon as this set of five flats had been inoculated, a second series exactly duplicating it was arranged. Thus inoculations on 500 plants, contained in ten flats, were made with the fresh virus. As will be seen in table III, which records the results of this experiment, the first series of five flats used for this fresh virus gave a total of 117 infections. The duplicate series gave 140. The average number of infections per set of 50 plants over the whole series was therefore 25.7 plants.

When the virus had stood at laboratory temperature for 24 hours a second similarly arranged test was made. Five hundred plants were again inoculated in exactly the same way, but with the one-day old virus. It was found that the number of infections had greatly changed, and that the two duplicate series agreed throughout in giving evidence of a decrease in strength. In five sets 50 infections had appeared. In the duplicate five sets 51 infections were found. The average number of infections per set of 50 plants was 10.1 plants, a much smaller result than was recorded for the fresh virus. If the fresh virus concentration be taken as 100 per cent, then it will be noted by reference to fig. 3, which represents the decrease due to dilution, that this one-day old virus acted as a 6

per cent suspension would be expected to do. If the reader desires to inquire into the accuracy of the duplicate series it will be possible to do so, using 4.0 for the probable error of a single comparison as shown in table II.

Before considering the behavior of this stored virus sample upon further aging, it must be mentioned that this experiment when repeated gives changed results according to the bacterial flora present and developing in the sample, and that this particular

TABLE III

EFFECT OF AGING ON EXTRACTED VIRUS AT 22° C.*

VIRUS	DUPLICATE DETERMINATIONS	OBSERVATIONS ON SERIES OF DILUTIONS					TOTALS	AVERAGES	STRENGTH INTERPRETED IN TERMS OF ORIGINAL (PERCENTAGE)
		Undiluted	1:4	1:8	1:16	1:64			
Fresh	1	35	29	29	16	8	117		
	2	36	36	28	28	12	140	25.7	100
One day old	1	22	11	11	2	4	50		
	2	20	9	11	8	3	51	10.1	6
One week old	1	42	41	29	35	19	166		
	2	44	36	27	36	21	164	33.0	400
Two weeks old	1	44	43	27	22	20	156		
	2	34	38	28	30	11	141	29.7	210
One month old	1	11	6	9	3	2	31		
	2	11	7	5	3	6	31	6.2	2

* The individual readings were made on a set of 50 test plants each time.

case is being recorded to indicate the actual findings in one instance. It is on the whole a good illustration of what happens to a virus at room temperature. Following the inoculations at the end of the first day a bacterial fermentation took place in the virus sample. Bubbles of gas rose through the liquid, and an odor like that of a fresh hay infusion was noted. This fermentation may have broken up solid particles in the suspension, thus liberating virus. Whatever its mode of action, a change in virus concentration was very evident when inoculations were again made at the close of a week. The first five sets of 50 plants, each inoculated in this seven-day sample, gave altogether 166 infections. The accompanying series

of five additional sets gave 164 infections. The samples for the duplicate series were all prepared independently, no one being made from another, but all from the undiluted stock bottle by the process of pipetting out known quantities of the virus and of water. The average number of infections per set for this one week test was 33.0 plants. This corresponds to the reading which would have been given by a sample four times as strong as the original. At the end of another week, fourteen days from the time when the fresh virus was tested, a new examination was made of the state of preservation of the virus, and it was found that two readings, 156 and 141, corresponding to the totals given before, were obtained. The average number of infections per set of 50 plants was therefore 29.7 plants. This indicated a strength still double that of the original, but lower than had been recorded at the end of the first week.

At the end of one month of storage the sample was again tested in the same way. The first 250 test plants gave 31 takes, the second 21 takes. It appeared then that a rather low level had been reached, the average number of infections per set of 50 plants being 5.2, and the strength of virus indicated being approximately 2 per cent of the original concentration. A subsequent test after four months indicated that a slow decrease had continued to take place. It must be remembered that this loss of 98 per cent of the strength after some weeks of standing does not mean that the virus would be considered very weak under some conditions, for when heavy inoculations are made as a regular practice, it is customary to dilute fresh viruses with water to such an extent that much weaker suspensions than this are obtained. It is possible to have 100 per cent of infections result from the use of these weak samples, because a large quantity of the virus is applied to the cut surfaces of the leaves. In many experiments, however, the loss of a large part of the virus strength is to be avoided, and in such work the results of this experiment indicate that speed of operation is a desirable feature.

Tests with frozen virus

At the end of a month and a half the sample of the original virus which had been frozen was melted and tested. It appeared that it had not lost as much of its strength as had the room temperature

sample. It had lost approximately 85 per cent of its effectiveness, however, as judged by comparison with the dilution chart. In the test which determined this, the average number of infections per set of 50 plants throughout the same series of dilutions as was reported in table III for the room temperature sample was 15.2, a reading lower than that given in the earliest test for the fresh virus by 10.5 plants. It is this difference which indicates the amount of loss due to the long standing while frozen. It will be seen, therefore, that the virus stored at room temperature was reduced to 2 per cent of its original strength in a month; that which was stored frozen retained 15 per cent of its strength as long as one and a half months.

When tests were made on the frozen fractions, which had been secured by thawing and pouring in such a way that one appeared to be clear ice water, one a deep brown low melting-point solution, and the third a mass of green débris suspended in a little fluid, it was found that the bulky ice fraction and the smaller débris-containing fraction had lost very little more of their strength than had been lost by the whole virus. At the same time the brown fraction had increased significantly in strength. Apparently the virus had been frozen out of the clear ice to a slight extent, and had followed the pigments and dissolved salts into the small volume of the low melting point fraction. Further work will be necessary before an unchanging virus can be secured by any method of storage. These experiments, illustrating the extent of the changes which may take place when a virus stands in a glass receptacle for some weeks, at least indicate that a virus does not retain all of its strength over long periods of time.

Green and yellow areas of mottled leaves

It is known that mottled leaves contain virus in the yellow areas and also in their apparently green areas. A careful examination of the green areas shows that they are sometimes invaded by yellowish patches, and that mistakes may arise in taking them all to be representative of the purest green areas available on green and yellow leaves. Preliminary tests showed that by loading the insect pin inoculating instruments with juice by sticking them

directly into tissues to be investigated and then into the leaves of healthy test plants, a great difference could be observed in yellow and green areas respectively as sources of virus. While the experiment recorded below does not show that the green areas are free from virus, it does show with certainty that the green areas are poor sources of virus as compared with the yellow areas immediately adjacent to them. In each of the ten parts of the experiment here described a single well marked leaf was taken, and examined first

TABLE IV

GREEN VS. YELLOW AREAS OF SINGLE MOTTLED LEAVES AS VIRUS SOURCES

LEAF NO.	YELLOW AREA	GREEN AREA	DIFFERENCE IN READING	DEVIATION FROM MEAN	DEVIATION SQUARED
1.....	30	11	19	1.2	1.4
2.....	16	6	10	7.8	61.5
3.....	21	0	21	3.2	10.0
4.....	33	2	31	13.2	174.0
5.....	28	6	22	4.2	17.6
6.....	21	1	20	2.2	4.8
7.....	29	5	24	6.2	38.4
8.....	12	2	10	7.8	60.8
9.....	12	1	11	6.8	46.2
10.....	12	2	10	7.8	60.8
			Mean = 17.8		Sum = 476

$\sigma = \frac{476}{10} = 6.9$ $Z = \frac{17.8}{6.9} = 2.58$ $n = 10$ Each reading was made on 50 test plants.

Odds for significance 10,000:1; odds that yellow area is at least eight times as strong a source of virus as green area about 100:1.

by making transfers to 50 plants from the green areas, then by making similar transfers from yellow areas to an equal number of plants. The leaves were not chosen as comparable, but merely as having well marked patterns. The yellow area readings are not all of the same order of magnitude, as will be noted in table IV, where they are summarized, but the difference between the yellow area on a leaf and the green area on the same leaf is consistently large, and always in favor of the thin yellow tissue as the better source of infective material.

The number of infections from the yellow areas was 17.8 higher on the average per set of 50 test plants than the number of infections

from the green areas. By reference to the dilution chart it will be noted that this corresponds to the difference as virus source between an undiluted and a 1:28 virus. The odds that the average difference of 17.8 represents a real difference between the two sources are very high, approximately 10,000:1. It appears, therefore, that *in mottled leaves* the virus distribution is indicated by the yellow pattern. It is known that leaves below the mottled ones on a plant are not free of virus after the plant has been infected for a long time, even though they are green. They may contain virus in almost as great a concentration as that in the yellow portions of mottled leaves, yet some may be almost free of virus, depending on their previous history and their position on the plant, as will be seen from the following account.

Spread of virus in inoculated plants

By inoculating a series of similar plants near their growing points, and then examining their leaves from time to time by making large numbers of transfers to healthy test plants, it was possible to assemble the data summarized in table V. This table shows that the green leaves above the point of inoculation quickly built up a concentration of virus comparable with that found in the yellow areas of mottled leaves, but that the green leaves below the point of inoculation remained for many days free of virus, later possessing a considerable amount. From the time of the appearance of the earliest symptoms, it is evident from the large numbers of infections secured in surveying leaves above the point of inoculation, that virus was present in the upper part of the plant. Only the column marked "developing leaf" has to do with leaves which show clearing of veins or mottling. The inoculated leaves never showed symptoms, yet they came to contain large amounts of virus. It seems necessary to assume from these figures that the larger part of the virus present in these green leaves below the mottled ones, but above the point of inoculation, was formed in them, and was not due to a backward flow from the developing leaves, because the original point of inoculation marked the limit of the virus-containing leaves. If the virus should flow back down the plant in quantity, it would not be expected to stop at the inoculated leaf. Yet virus was found, from the

time of the appearance of the earliest symptoms until the end of three weeks from the time of inoculation, to be present in large amounts in the inoculated leaf and those above it, and to be absent from the leaves immediately below. The later development of virus in the lower leaves must indicate a migration of at least a small amount of virus backward down the plant.

TABLE V

SURVEY FOR SPREAD OF VIRUS*

	LOWEST GREEN LEAF	INTERMEDIATE LEAF (BELOW)	ORIGINALLY INOCULATED LEAF	INTERMEDIATE LEAF (ABOVE)	DEVELOPING LEAF
	Inoculated when 8 inches high				
Examined at:					
Clearing of veins	0	0	29	43	42
First mottling	1	0	25	45	39
Three weeks	0	0	32	37	36
Four weeks	49	47	42	46	5
Five weeks	10	29	40	42	40
	Inoculated when 1 inch high				
One week	.....	.....	11	30	24
Two weeks	.....	.....	28	38	42
Three weeks	.....	.....	35	48	43
Four weeks	.....	.....	45	39	43
Five weeks	.....	.....	33	44	44

* Each number in the table here given represents the results of the inoculation of 50 test plants. The exact reading should not be considered important, since there is some variation when as few as 50 plants are used in each test. The groups of figures are consistent enough, however, to show the trend of virus concentration in the leaves in each position considered.

It seems necessary to conclude that the virus may develop and come to high concentration in leaves which appear very different, some being green, others mottled; and that mottling is not so much an indicator of the presence of virus as it is an indication that virus was present at the time the leaf developed. More careful studies will be made later. This survey indicates in a general way the behavior of the virus as it spreads through the plant. In addition to the points of theoretical interest involved, the survey throws some light on the length of time plants should stand infected before their leaves are used as sources of virus. It is hoped that the account here given of the method used for obtaining concentration readings on tobacco mosaic virus may be of value to other investigators who

would like to know the strengths of the viruses with which they may work, and what degree of reliance may be placed on readings of such strengths.

Summary

1. A method of inoculating test plants with small, uniform doses of tobacco mosaic virus is described. The object of the method is to lessen the mechanical difficulties usually met with in the attempt to obtain reliable quantitative results. By the use of small plants, inoculated by pricks of insect pins held in a convenient handle, very large numbers of test inoculations may be performed with economy of time and effort, and with a minimum of greenhouse space and practically no danger of contamination from handling.

2. It is shown that dilution to a given extent causes a decrease in infections when inoculations are made into test plants, and that this decrease is not dependent upon the original concentration of the virus, but only upon the percentage dilution in the range studied. Charts and a graph are given so that the results with known dilutions may be available for grading unknown samples of virus. It is shown that the numbers of plants required for significant results in proposed experiments may be predicted.

3. Experiments are described showing that by the use of such methods it can be demonstrated that virus tends to die off rather rapidly in storage at 22° C., more slowly but still considerably when frozen; that the yellow areas of mottled leaves are much better sources of virus than the adjacent green areas of the same leaves; that green leaves above the point of inoculation may quickly become strong sources of virus, yet apparently similar green leaves immediately below the point of inoculation remain free of virus for some weeks, later becoming effective sources of virus.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.
YONKERS, N.Y.

[*Accepted for publication March 24, 1928*]

FURTHER OBSERVATIONS ON STRUCTURAL DEFECTS OF THE GRAFT UNION

E. L. PROEBSTING

(WITH SIX FIGURES)

A preliminary survey of the structure of the graft union has been made, designed to give an indication of the types of unions to be expected, and some idea of whether or not correlation between field behavior and structure could be observed. Since the California Agricultural Experiment Station is engaged in extensive field trials of grafted material, a considerable number of combinations was available for study, in addition to specimens of various kinds and different ages from other sources. The Experiment Station material was largely one-year-old budded nursery stock. From one to six unions of each of thirty-one combinations were sectioned.

A brief description of a type of abnormality, consisting essentially of a double layer of bark accompanied by a considerable amount of wood parenchyma between stock and scion, was published in 1926.[1] The studies of which those observations formed a part have been extended to other interspecies grafts of *Pyrus*, and to several combinations of *Prunus* species.

Exceedingly great variations occur in the structural details at the line of union of stock and scion. The range may be said to extend from the combination which forms a perfect union, and which exhibits no defects of any sort, to the extremely uncongenial pair which does not form a union of any sort, and which results in the death of the scion. Just short of this latter extreme is the type just mentioned, of bark formation between stock and scion in which the scion may start growth, but in some cases may not live through the season. This type of union is found in some combinations of *Prunus* as well as in *Pyrus*, notably the Beauty plum (*Prunus salicina*) on almond (*P. communis*), and in the Italian prune (*P. domestica*) on the sweet cherrry (*P. avium*). The former combination is illustrated

[1] PROEBSTING, E. L., Structural weaknesses in interspecific grafts of *Pyrus*. BOT. GAZ. 82:336–338. 1926.

in fig. 1. In this example there is a segment of the union that is nearly normal.

The normal union may be considered to approach the type shown by prune (*P. domestica*) on Myrobalan (*P. cerasifera*). In transverse sections of budded material the wood of the stock next the cambium is ordinarily summer wood. When the bud begins growing spring wood is the first laid down. The effect produced, therefore, is essentially similar to that produced by the adjacent summer and spring wood of two annual rings. The medullary rays are continuous across the line of union. The pits in adjacent cells meet as though the cells were derived from a single source. The cambium cells of the scion have formed medullary ray cells if they were in contact with ray cells, and xylem elements if they were in contact with xylem elements. It seems a curious circumstance that the cells of the stock should exert this influence on the differentiation of cells of the scion. This condition was found in all unions which did not exhibit some other type of structural defect, and in some unions which did.

FIG. 1.—Beauty plum on almond, showing layers of bark extending part way across union; center of union sound.

The line of union in a transverse section of a normal union can usually be identified by differences in character of tissue or cell structure. Gross differences in the appearance of the two tissues, which can be seen without magnification, may sometimes serve as a means of orientation. It is often practically impossible to identify the last cell of one sort and the first of the other in sectioned material, even when the general region of the union is well defined.

In radial sections of budded material, the line of union is usually indicated by a sharp angle between the axis of the stock and that of the scion at the point of union. The bud (scion) having been inserted on the side of the stock, its first growth is in the same direc-

tion as that which a lateral bud would make. After that part of the stock above the bud has been cut off, and the scion has grown over the cut surface, the growth rings in a normal union tend to form continuous cylinders, with the line of union becoming nearly transverse. This is shown diagrammatically by WAUGH.[2] With grafted material, the surface of contact between the wood of the stock and of the scion which had differentiated before the grafting took place, and which never unite, can be used as a means of orientation. Radial sections show the continuity of rays across the line of union as well as do transverse sections. Fig. 2 is an example.

The tangential section, especially in older specimens where the line of union has become nearly transverse in the younger wood, is usually the most striking, and shows abnormalities in the clearest manner. Differences in the character of the rays, vessels, or fibers are shown very well. In good unions the continuity of tissue is again shown by the way in which the xylem elements are dovetailed together to make a well knit, mechanically strong juncture. A section of this sort is shown in fig. 3. In poor unions the defects stand out sharply, as will be shown later.

Perhaps the most common type of structural defect is that involving the deposition of wood parenchyma by the cambium at the line of union, interrupting the vascular connection to a considerable extent. This may be seen in any plane. There is a great deal of variability in the extent to which this condition may be expressed. In some cases there may be only isolated masses of parenchyma with the vessels making fairly good connections around them. In others the ends of the vessels may be almost entirely separated by these cells. In extreme cases the vascular tissue adjacent to the parenchyma may show distortion, and the layer of parenchyma be continuous. It appears that the cambium of the stock, of the scion, or of both, adjacent to the line of union produces only parenchyma cells, giving practically a continuous sheet of these cells between stock and scion. The result of such a condition is a mechanically weak structure which may survive several seasons, but which will ultimately break off as the increased leaf surface of the top offers more

[2] WAUGH, F. A., The graft union. Mass. (Hatch) Agric. Exp. Sta. Tech. Bull. 2. 1904.

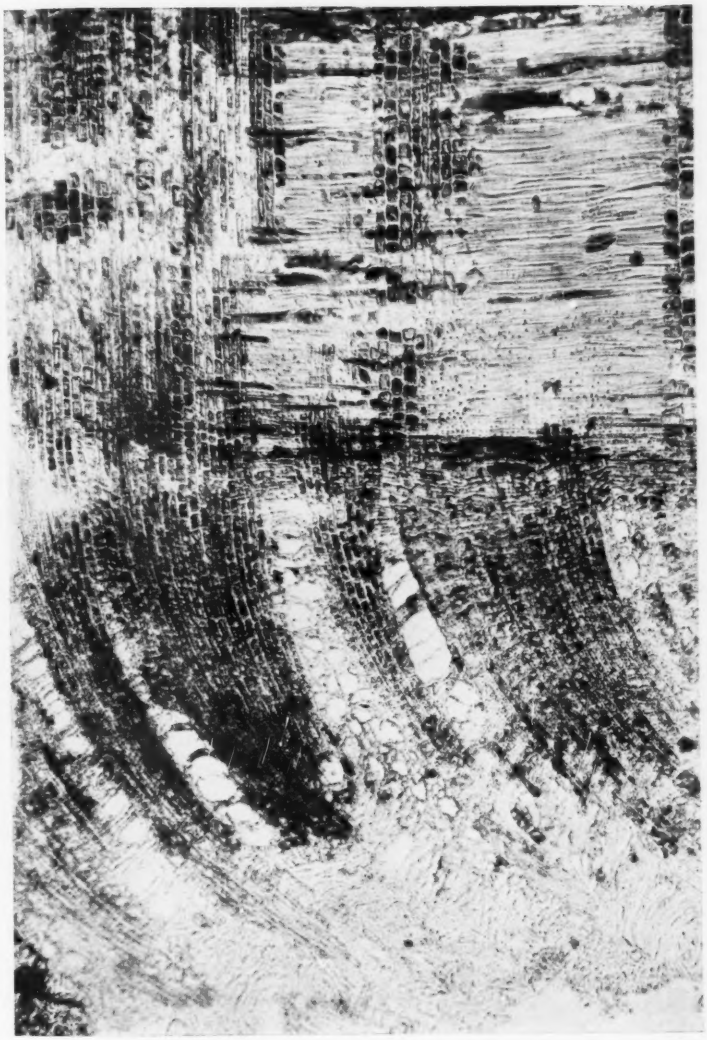


FIG. 2.—Radial section of union of Formosa plum on Myrobalan, showing change of plane of scion and continuity of rays across union.

resistance to the wind. Fig. 4 shows such a union which has broken off. This condition occurs in such unions as apricot on Myrobalan, which often breaks off in this manner, and in many other combina-

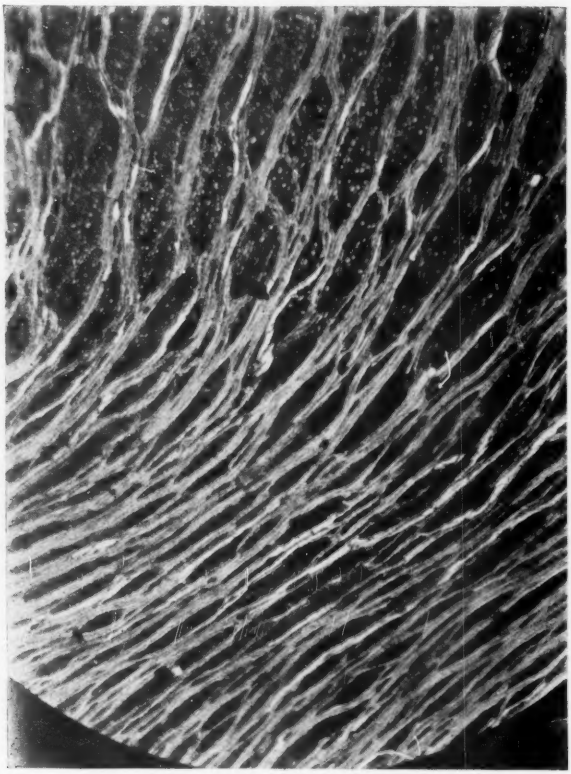


FIG. 3.—Tangential section of union of French prune on Muir peach, showing dovetailing of vascular elements of stock and scion; difference in size of rays identifies the two tissues.

tions in a less marked degree. This is shown in tangential section in fig. 5.

Distortion of the vascular tissue, while it may not cause either mechanical weakness or functional disturbance in mild cases, is commonly encountered in the neighborhood of the union. This distor-

tion may be confined to the formation of a sharp angle of xylem and of phloem elements, in the case of a stock which grows much more rapidly or more slowly than the scion. Almond on peach frequently shows this sharp angle at the line of union. It can be seen by inspection with the naked eye, as well as by the examination of sections.

FIG. 4.—Result of parenchyma formation at line of union of apricot on Myrobalan.

It has no apparent influence on the life of the tree or its behavior. BRADFORD and SITTON[3] have emphasized this point with regard to the apple. However, when the distortion reaches the degree of forming whorls and loops, and of showing vessels in both longitudinal and transverse section within the space of a millimeter, and when

[3] BRADFORD, F. C., and SITTON, B. G., Some cases of false uncongeniality in graft unions. Proc. Amer. Soc. Hort. Sci. 24: 1927 (in press).

the continuity of the vascular system appears to be definitely broken by these contortions, it is difficult to believe that there is not a functional disturbance as a result. Keeping in mind the danger of drawing conclusions bearing on function merely from observations on

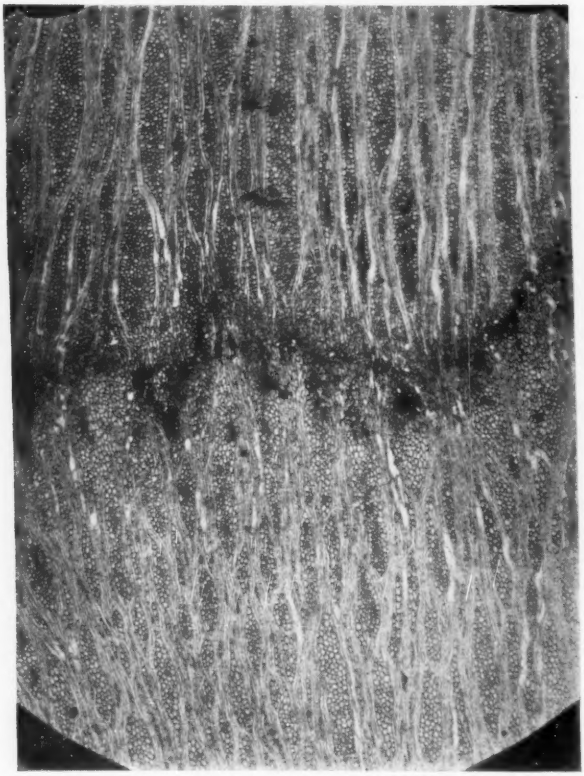


FIG. 5.—Tangential section of union of Phillips peach on apricot, showing wood parenchyma interrupting vascular continuity.

structure, and the fact that distortion is a normal feature of such structures as the nodal plates of corn, it should be recognized that some other factor may be of greater importance in governing the

behavior of such combinations in the field. These seriously distorted regions are sometimes associated with masses of parenchyma, and at times with the degeneration of certain areas to amorphous, gummy masses. The occurrence of such a condition is illustrated in fig. 6.

Another type of structural defect is found in some combinations of *Prunus*. In this type all of the xylem between the medullary rays at the line of union is degenerated into a gummy mass. These gum pockets are continuous around the medullary rays and may extend for considerable distances. Obviously, unless the region of gumming is confined to a portion of the xylem at the union, water movement would be expected to be very materially checked. If the area affected is small in comparison with the total surface of the union its effect would be small. The condition described may occur at the line of union only by chance, and not be an effect of the interaction or lack of interaction of the grafts. A very similar condition may occasionally be seen in limited areas in other locations than at the line of union. It is possible, therefore, that the gum formation is a reaction to some other stimulus than that induced by the graft.

The preceding discussion has been confined to conditions obtaining in the wood. The bark exhibits abnormalities fully as great. Both the intervention of cork layers between the phloem of the stock and scion and distortion comparable with that described have been noted. It is conceivable that interference with translocation may be one of the major factors in the failure of certain combinations to make satisfactory unions. Irrespective of the various theories of the path of translocation of foods and nutrients, there is adequate evidence that in certain unions the movement across the union would be expected to be difficult. That such an interference does exist is supported by the occasional appearance of swellings above the union, such as would be caused by ringing.

The observations recorded here are in no sense to be considered a complete inventory of the structures to be observed in grafted material, even of the combinations considered. From the limited number of grafts of a single combination sectioned, it is evident that there is a high degree of variability to be found between individual

specimens of a given combination. It would seem that an extensive study of the variations encountered in a single combination, with a

FIG. 6.—Distortion of tissue accompanied by gum formation at union of Japanese plum on almond; tangential section.

concurrent search for the factors inducing the variations, would prove profitable.

The combinations which have served as the basis of these notes are the following:

PRUNUS.—On Myrobalan (*P. cerasifera*):
- Orange Cling and unknown peaches (*P. persica*)
- Blenheim apricot (*P. armeniaca*)
- Sugar and Imperial prunes (*P. domestica*)
- Tragedy plum (*P. domestica*)
- Beauty, Santa Rosa, and Formosa plums (*P. salicina*)

On apricot (*P. armeniaca*):
- Santa Rosa plum (*P. salicina*)
- Sugar prune (*P. domestica*)
- Palora, Phillips, and Muir peaches (*P. persica*)

On Muir peach (*P. persica*):
- Beauty plum (*P. salicina*)
- I.X.L. almond (*P. communis*)
- French prune (*P. domestica*)

On almond (*P. communis*):
- Unknown almond
- Beauty plum (*P. salicina*)

On Sweet cherry (*P. avium*):
- Italian prune (*P. domestica*)

PYRUS.— On apple (*P. malus*):
- Apple
- Bartlett pear
- Quince

On French pear (*P. communis*):
- Bartlett pear
- Unknown apple

On Japanese pear (*P. serotina*):
- Bartlett pear
- Quince
- Apple

specimens of a given combination. It would seem that an extensive study of the variations encountered in a single combination, with a

FIG. 6.—Distortion of tissue accompanied by gum formation at union of Japanese plum on almond; tangential section.

concurrent search for the factors inducing the variations, would prove profitable.

The combinations which have served as the basis of these notes are the following:

PRUNUS.—On Myrobalan (*P. cerasifera*):
- Orange Cling and unknown peaches (*P. persica*)
- Blenheim apricot (*P. armeniaca*)
- Sugar and Imperial prunes (*P. domestica*)
- Tragedy plum (*P. domestica*)
- Beauty, Santa Rosa, and Formosa plums (*P. salicina*)

On apricot (*P. armeniaca*):
- Santa Rosa plum (*P. salicina*)
- Sugar prune (*P. domestica*)
- Palora, Phillips, and Muir peaches (*P. persica*)

On Muir peach (*P. persica*):
- Beauty plum (*P. salicina*)
- I.X.L. almond (*P. communis*)
- French prune (*P. domestica*)

On almond (*P. communis*):
- Unknown almond
- Beauty plum (*P. salicina*)

On Sweet cherry (*P. avium*):
- Italian prune (*P. domestica*)

PYRUS.— On apple (*P. malus*):
- Apple
- Bartlett pear
- Quince

On French pear (*P. communis*):
- Bartlett pear
- Unknown apple

On Japanese pear (*P. serotina*):
- Bartlett pear
- Quince
- Apple

On *P. ussuriensis:*
Bosc pear
Bartlett pear
On quince (*Cydonia oblonga*):
Bartlett pear

The writer wishes to acknowledge his indebtedness to Mr. M. J. HEPPNER for his cooperation in supplying many of the unions sectioned, and to Dr. W. P. TUFTS for valuable suggestions in the preparation of these notes.

COLLEGE OF AGRICULTURE
DAVIS, CALIF.

[*Accepted for publication February 13, 1928*]

CHANGES IN SIZES OF YEAST CELLS DURING MULTIPLICATION

OSCAR W. RICHARDS

(WITH FIVE FIGURES)

During the period of rapid proliferation in the early growth of a population of yeast in test tube culture the cells are of nearly uniform size. As the toxic excretion products accumulate the larger buds are selectively injured, and the population is then composed of large resistant cells and small attached buds. In an effectively constant medium the distribution of cells of different sizes is unimodal. The changes in the sizes of cells are described in detail in this paper.

The diffused nucleus of yeast[1] prevents accurate measurement of the size ratio of the nucleus to the cytosome, so that it is not possible to determine the relation between this ratio and the initiation of budding. The writer's measurements show that the process of bud formation bears no relation to the size of the mother cell. The method proposed by BECKING[2] for the analysis of the size changes of cells during increase is not applicable to the measurements of yeast without great modification. SLATOR[3] followed the increase of yeast on a warm slide, and CLARK[4] photographed the cells, but neither measured the size of the individual cells.

I

Two different strains of *Saccharomyces cerevisiae* were used in these experiments, and they will be referred to by the name of the

[1] The investigations on the histology of the yeast cell are summarized in GUILLIERMOND, A., Les Levures. Paris. 1912.

[2] BECKING, L. B., and BAKER, L. S., Studies on growth. Stanford Univ. Publ. Biol. Sci. 4:135. 1926.

[3] SLATOR, A., Some observations on yeast growth. Biochem. Jour. 12:248–259. 1918.

[4] CLARK, N. A., Rate of formation and yield of yeast in wort. Jour. Physical Chem. 26:42–60. 1922.

place where each was used, as Oregon data and Cambridge data.[5] The origin of the yeast and the technique used in measuring growth have been described.[5] The cultures were kept at 30°±0.8° C. in an incubator. This is the customary temperature for yeast culture; but evidence, to be published later, indicates that if this temperature of 30° is maintained within a few hundredths of a degree, it is critical for the budding of yeast. The latitude of variation of the usual laboratory incubators is so great that the cultures are not kept at the critical thermal point long enough for the critical effect to influence the growth to an appreciable extent.

The measurements were made with a planimeter from tracings of the outlines of the cells. Photomicrographs were enlarged with an Edinger drawing apparatus to give a total magnification of three to four thousand times before they were traced. The cells are almost spherical, so that a sharp image is formed only when the greatest diameter is in focus. Only those cells whose photographs were sharp were measured. The measurements are given in arbitrary area units, and the volumes of the cells are almost proportional to the three-halves power of the area measurements. The error of projection and measurement is about 5 per cent, as determined by making two independent measurements of the same group of cells.

The photomicrographs were made by placing a pocket kodak on top of the microscope. The mounting of the f. 6.9 lens just fits within the rolled edge of the ocular and makes a light-tight mounting. The difference in focus between the eye and the film was corrected for with a −9 diopter lens.[6] The yeasts were photographed in a haemacytometer, so that the ruling would indicate the magnification. The magnification of the image on the kodak film was about 150× when the 4 mm. objective and a 10× ocular were used. In the later experiments the advantage of the autographic feature was sacrificed

[5] RICHARDS, O. W., The growth of the yeast *S. cerevisiae*. The growth curve, its mathematical analysis and the effect of temperature on the yeast growth. Ann. Botany 42:1–14. 1928.

———, Potentially unlimited multiplication of yeast with constant environment and the limiting of growth by changing environment. Jour. Gen. Physiol. 11:525–538. 1928.

[6] Using this suggestion from FOOT and STROBEL, quoted in GUYER's Animal micrology. Chicago. 1921 (p. 150).

for the greater ease of handling and the less expense of moving-picture film. A suitable insert reduced the opening in front of the film to twice the size of the standard movie exposure.

II

The changes in the size of the cells during budding were followed by placing a few cells in nutrient fluid in the counting chamber and sealing the edges with vaseline. The cells were kept in an incubator at $30° \pm 0.8°$ C., except for the two minutes necessary to photograph them at the end of each 30-minute period. The cultures grew well and no unfavorable changes were observed before nearly 24 hours. Seven groups of cells were measured, each containing 2–11 cells.

The changes in size of a typical group of cells are shown in fig. 1. The growth, or increase in size, is made by the buds. The changes of the cells which are not budding are on the average less than the error of measurement and are not significant.

The average decrease in average cell size in the 30-minute interval preceding bud formation is 15.8 per cent. The cell giving off the bud decreased, on the average, 15.5 per cent in area units during the same interval. This shows that other cells which remain attached together in the group are not involved during the formation of the bud. During the same interval the growth increment in total area of all of the cells in the group was found to be about 8 per cent. The unaccounted-for size of the bud amounts to an average of 72 per cent, which must come largely from the imbibition of water.

BECKING has devised a method of studying cell size changes, assuming the cell growth to be a discontinuous process. These changes he describes objectively with tri-dimensional graphs which he calls clivoids. A clivoid showing the distribution of the sizes of the yeast group of fig. 1 is shown in fig. 2. This clivoid shows two growth periods without cell division, and is not comparable directly with any of the cases in BECKING'S paper. His class VII, for cell division without cell growth, under unbalanced conditions, most nearly approaches the observations on yeast. His treatment could be extended to include the present observations for growth of the bud and not of the mother cell. The labor involved in the further mathematical development does not seem justified until more measurements of the changes in cell size are available.

III

The average size of the cells present during the growth of a population of yeast follows a regular cycle. The average size decreases

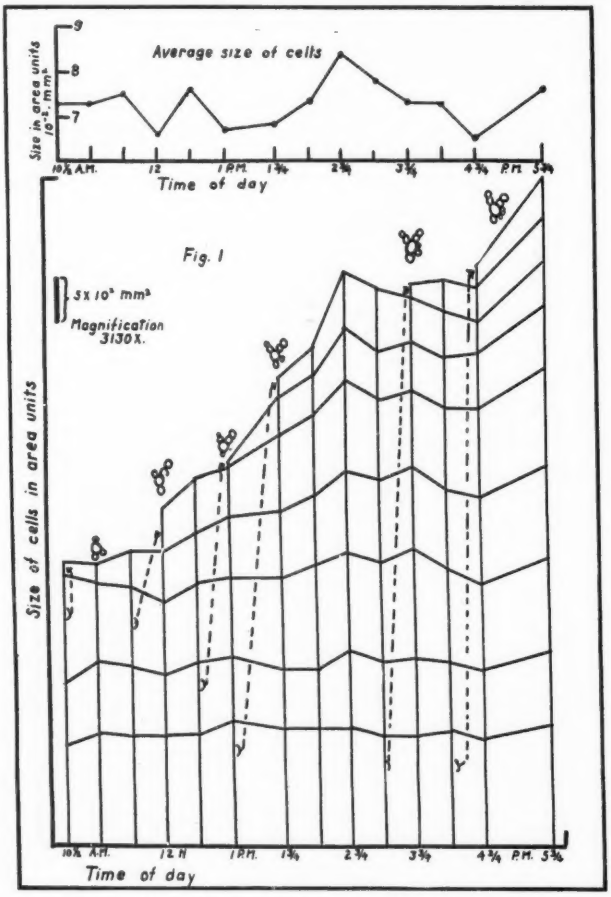


FIG. 1.—Changes in cell size during growth of a group of yeast

during the first 20 hours' growth after seeding, and then increases. The smallest size is found at the time that cell proliferation is greatest. The curves of average size are similar for the two strains meas-

ured, as shown in figs. 3*a* and 3*b*. Each point in fig. 3*a* is the average of ten measurements, that of fig. 3*b* is the average of thirty measurements, and each point in fig. 3*c* is the average of twenty-five measurements. This cycle of size change is not significantly altered if the acidity of the medium is maintained almost constant, but it is different if the environment is kept effectively constant in other respects (fig. 3*c*).[7] The asymmetry of the cycle is probably due to the changes in the environment produced by the accumulation of excretion products.

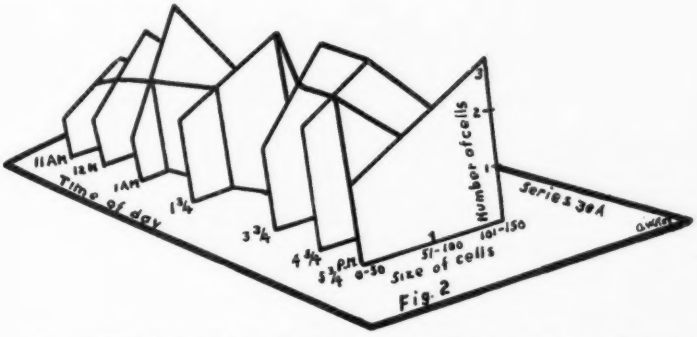


FIG. 2.—Clivoid showing distribution of sizes of cells shown in fig. 1, with respect to time.

The distribution of the sizes of cells present at different times is shown in figs. 4 and 5. In fig. 4 the average cell size (cf. fig. 3*a*) is indicated by the partition extending along the time axis. The distribution of the cells comprising the seeding depends on the age of the culture. During the rapid growth of the first 20–30 hours, more smaller cells are present and the distribution is practically unimodal. As the rate of growth of the population is gradually retarded by the accumulation of excretion products, the distribution becomes bimodal. This is most pronounced at about 100 hours after seeding, when the population is just maintaining itself at an equilibrium number.

The description of the yeast clivoids suggests a recurrence of cell

[7] Compare the growth curves given in[6] fig. 4*a*. The September observations of this figure are on the cells measured for figs. 3*c* and 5 of the present paper.

sizes such as would be expected with discontinuous growth, which BECKING emphasizes and which resembles his case III, when growth is a function of and dependent on cell size. The asymmetry of the

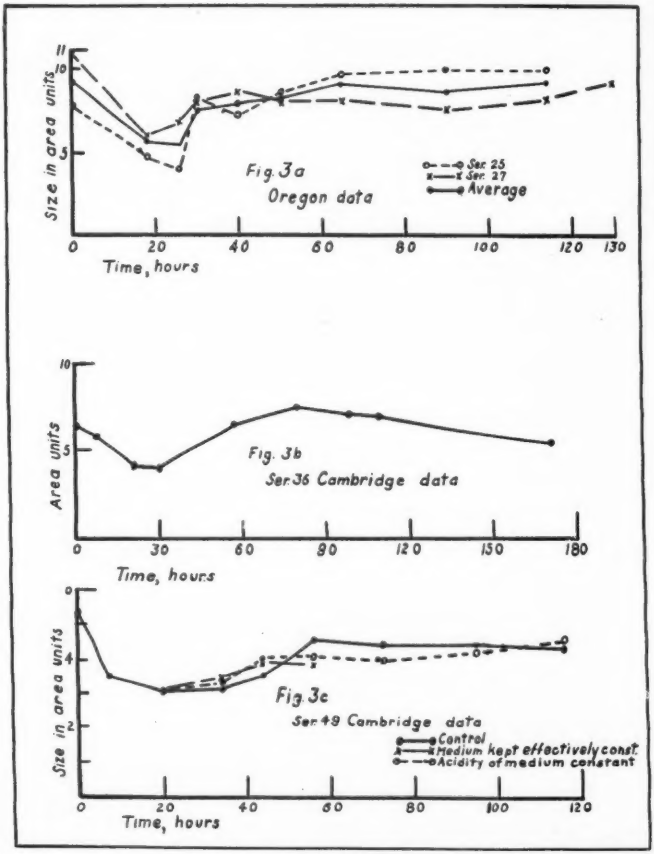


FIG. 3.—*a*, average size of cells (Oregon data); *b*, average size of cells (Cambridge data, series 36); *c*, average size of cells (Cambridge data, series 49).

recurrence suggests that it is not fair to apply the theory unless the effects due to changing environment are avoided. The tri-dimensional graphs indicate objectively the accumulation of these environmental effects.

The number of cells budding at the time of most rapid growth of the population is 22 per cent. At the period of equilibrium growth, about 100 hours, this is only reduced to 19 per cent. The difference is hardly significant, and shows that the mature cells are able to resist the toxic changes in the environment and to continue

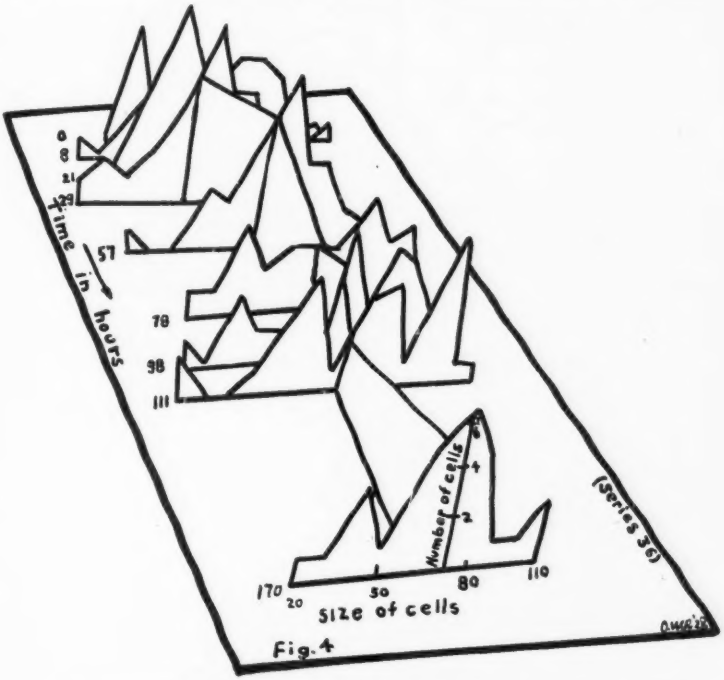


FIG. 4.—Clivoid showing distribution of sizes of cells (Oregon data, series 36)

to bud actively. The larger buds are selectively affected, and are apparently destroyed as soon as they are independent of the mother cell and before they are able to acquire resistance. This seems to be the explanation of the bimodal size distribution at this time. Much later, at 170 hours after seeding, budding practically ceases and the distribution of sizes again becomes unimodal.

The distribution of sizes of cells present in the population grown in a medium maintained at an almost constant acidity does not

depart significantly from that characteristic for the control population (fig. 5). The population grown in an effectively constant environment contains a unimodal distribution of cells until nearly the time that the experiment must for technical reasons be discontinued. The bimodality of the last time measured, 56 hours, is due apparently to a slight accumulation of waste products that has not reached a sufficient concentration to cause a decreased rate of multiplication. This probably accounts for the slight decrease in the average size of the cells (fig. $3c^8$). Up to this last observation there is no indication of a cyclic change in the average cell size.

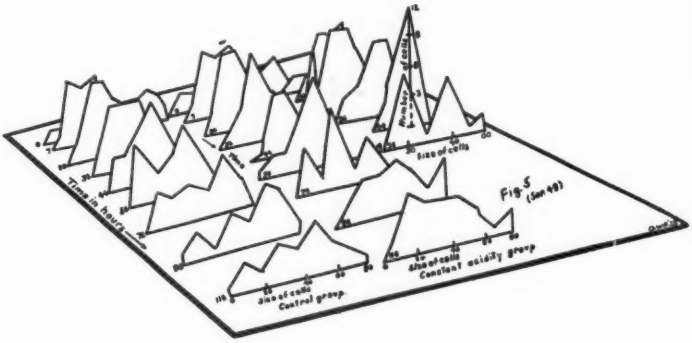


FIG. 5.—Clivoid showing distribution of sizes of cells (Cambridge data, series 49)

The distribution of the sizes of yeast growing in an effectively constant environment at a constant rate does not fit in any of the cases proposed by BECKING. These experiments suggest that the recurrence of the size modes of the cells is due to changes in the environment rather than to the fact that the cells regularly pass into the next larger size group, as would be the case if cell division were a direct result of cell size. Unfortunately, the technical difficulties prevented keeping the cultures in an effectively constant medium for a longer time. These observations give no indication that budding is a function of the size of the cells. Budding seems to be determined by the physiological condition of the larger cells, which are less affected by changes in the culture medium.

Summary

1. The changes in the distribution of the sizes of cells with time are described for the growth of a population of yeast.

2. These changes follow a definite cycle, which is associated with the accumulation of toxic excretion products in the culture medium. When the medium is kept effectively constant the cycle does not occur.

3. The method proposed by BECKING, for the analysis of growth as determined by the size of the cell that is about to divide, is shown not to be applicable, without fundamental modification, to the analysis of the growth of yeast.

4. Bud formation is independent of the size of the mother cell, and seems to be determined by the physiological condition of the larger cells, which are less affected by changes in the culture medium.

LABORATORY OF GENERAL PHYSIOLOGY
HARVARD UNIVERSITY

[*Accepted for publication February 12, 1928*]

EFFECT OF SUNLIGHT ON SAP CONCENTRATION OF CITRUS LEAVES[1]

F. F. HALMA AND A. R. C. HAAS

DIXON and ATKINS (2) and CHANDLER (1) have pointed out the effect of sunlight upon the sap concentration of leaves of different species of plants. The samples of citrus leaves employed in previous work of HAAS and HALMA (3) were collected from the shady side of the trees, in order to avoid effects of intense sunlight upon the sap concentration. The usual method of gathering leaf samples from trees has been to collect leaves from any or all sides of the trees. In so doing, leaves have been obtained some of which were exposed to direct sunlight, while others were in dense shade. That a large error would result from such a procedure will be shown in this paper. Moreover, evidence will be presented to show that Eureka lemon and Valencia orange leaves respond differently to the photosynthetic process, and that a relation exists between this phenomenon and the anatomy and chemical make-up of the types of leaves used.

The material consisted of normal mature leaves, one or more years of age, collected from ten-year-old trees growing on the grounds of the Citrus Experiment Station, Riverside, California. At each sampling, two sets of leaves from each tree were taken, one on the shady and the other on the sunny side. In choosing leaves on the sunny side, care was taken to select leaves which were fully exposed to direct sunlight. On the shady side only leaves from dense shade were selected. The leaves were picked before 9:30 A.M., and as quickly as possible, the greatest interval in time between the first and the last sample never exceeding twenty minutes. As an additional precaution, the order of taking the samples was varied at each sampling. The preparation of the material and the determination of the sap concentration were the same as in previous work by HAAS and HALMA (3).

[1] Paper no. 180, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

From table I it is clear that when the material is secured from the shady side, the lemon leaf sap is considerably more dilute than the orange leaf sap. Similar but much smaller differences were found in sap of leaves obtained from the sunny side. Moreover, it was found that on prolonged exposure to sunshine the differences in sap concentration between lemon and orange leaves may disappear entirely. Since the leaves of the two species were practically identical in maturity, we may infer that either the lemon leaf is more efficient in its rate of building up of photosynthetic products than the orange, or the rate of translocation or respiration in the orange leaf is greater than in the lemon leaf. That the increase in freezing point lowerings, brought about by the action of direct sunlight, is due to carbohydrate metabolism is seen in the fact that the ash content of the expressed sap remains virtually the same, regardless of whether the leaves were in shade or in sunshine.

The question arises as to whether the exposure to direct sunlight increases the rate of transpiration and thereby lowers the moisture content of the leaves, and consequently increases the sap concentration. Again this can hardly account for the great differences found, since the ash content (table I) is not affected by exposure to direct sunlight.

We may now return to a previous statement, to the effect that either the lemon leaf is more efficient in the building up of metabolic products than the orange, or the former has a slower rate of translocation than the latter. We have evidence tending to give support to the former view.

In cross-sections of lemon and orange leaves (52 each), of the type used in sap concentration studies, the percentage of the width of the section occupied by the palisade tissue was measured and the following values were obtained:

	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
Eureka lemon leaves	29.4±0.38	2.82±0.27	9.59±0.92
Valencia orange leaves	23.8±0.31	2.30±0.22	9.66±0.93

It is evident that, at least in the type of leaves used in this work, the palisade cells of the lemon leaf occupy more space than is the case with the orange leaf. Since the palisade tissue is the chief photosynthetic unit of the leaf, this fact strengthens the view that the

TABLE I

EFFECT OF SUNLIGHT UPON CONCENTRATION AND ASH OF LEAF SAP

DATE 1927	EUREKA LEMON					VALENCIA ORANGE					INCREASED FREEZING POINT LOWERINGS OF VALENCIA ORANGE OVER EUREKA LEMON (° C.)	
	Δ		Difference (° C.)	Ash of 20 cc. sap		Δ		Difference (° C.)	Ash of 20 cc. sap			
	Shade	Sun		Shade	Sun	Shade	Sun		Shade	Sun	Shade	Sun
September 13	1.387	1.592	0.205	.....	.....	1.631	1.681	0.050	.....	.....	0.244	0.089
16	1.359	1.590	0.231	.....	.....	1.801	1.911	0.110	1.5288	1.5284	0.442	0.321
20	1.317	1.568	0.251	0.7306	0.7136	1.727	1.783	0.056	.....	.....	0.410	0.215
23	1.327	1.422	0.095	0.6262	0.6123	1.694	1.741	0.047	1.4005	1.4031	0.367	0.319
26*	1.160	1.513	0.353	.....	.....	1.566	1.696	0.130	.....	.....	0.406	0.183
October 13	1.334	1.566	0.232	.....	.....	1.498	1.614	0.116	.....	.....	0.164	0.058
November 3	1.187	1.387	0.200	.....	.....	1.397	.....	.....	.....	.....	0.210	.....

* Mature leaves of current season from same trees as samples of September 20.

lemon leaf is more efficient photosynthetically than is the orange leaf, provided the rates of translocation and respiration are similar in both.

It has been shown by HAAS and HALMA (3) that the leaf sap of the Eureka lemon contains more magnesium than that of the Navel or Valencia orange. In view of the fact that magnesium is a constituent of chlorophyll, differences in magnesium content of the sap may possibly be another index of the photosynthetic activity of the leaf. Further indirect evidence is furnished by the fact that leafy lemon cuttings root much more rapidly than similar cuttings from orange (4). In addition, unpublished data of HALMA bring out the fact that leaf cuttings of the lemon produce nearly twice as much dry weight of roots per unit weight of leaf as orange leaf cuttings, when grown under the same conditions. Investigations now under way indicate a relation between the percentage of the leaf cross-section occupied by palisade tissue and rooting ability of leafy cuttings of the species; leaves having greater percentages of palisade tissue root more readily. It is realized that the respiratory, photosynthetic, and translocatory processes are interrelated, hence further discussion on leaf efficiency requires additional data.

In conclusion, it is well to emphasize the importance of collecting samples under uniform conditions of illumination. This precaution does not apply only to sap concentration studies, but equally well to determination of photosynthetic products. Large errors are bound to occur if the important effects of differences in exposure to sunlight, here pointed out are ignored.

Summary

1. Increases in the sap concentration of leaves of Eureka lemon and Valencia orange occur when the leaves are exposed to direct sunlight.

2. The sap of Eureka lemon leaves on exposure to direct sunlight increases its concentration more rapidly than does that of Valencia orange leaves.

3. Increases in sap concentration due to the action of sunlight on the leaves are due entirely to photosynthetic products, the ash of the sap remaining the same under both conditions.

4. A greater percentage of the cross-sectional diameter of the Eureka lemon leaves is occupied by palisade tissue than is found to be the case with the Valencia orange leaves. This, together with the fact that more magnesium is found in the sap of Eureka lemon leaves than in that of Valencia orange leaves, and the more rapid response of leafy lemon cuttings to vegetative propagation, lends support to the view that greater increase in lemon leaf sap concentration in direct sunlight over that of orange leaf sap may be due to a more efficient photosynthetic system.

5. The great variation in sap concentration of citrus leaves brought about by direct sunlight indicates that large errors may result through indiscriminate sampling.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIF.

[*Accepted for publication January 12, 1928*]

LITERATURE CITED

1. CHANDLER, W. H., The killing of plant tissue by low temperature. Mo. Agric. Exp. Sta. Res. Bull. **8**: 143–309. 1913.
2. DIXON, H. H., and ATKINS, W. R. G., Variations in the osmotic pressure of the sap of the leaves of *Hedera helix*. Sci. Proc. Roy. Dublin Soc. **13**:239–246. 1912.
3. HAAS, A. R. C., and HALMA, F. F., Physical and chemical characteristics of expressed citrus leaf sap and their significance. BOT. GAZ. **85**:457–461. 1928.
4. HALMA, F. F., Propagating citrus by cuttings. Calif. Citrograph **11**:225. 1926.

COLEOPTILE OF ZEA MAYS AND OTHER GRASSES

GEORGE S. AVERY, JR.

(WITH TEN FIGURES)

Whether the coleoptile in grasses is to be interpreted as a single leaf, or as the result of the union of two organs, is still a matter for discussion. SARGANT and ARBER[1] favor the theory that the coleoptile is the equivalent of a pair of fused stipules. WORSDELL[2] substantially agrees, concluding that it is a ligular-like structure formed by the union of stipules. They base their interpretations principally on the presence of the two vascular bundles characteristically present in the coleoptiles of most grasses. These two bundles establish a double symmetry in the coleoptile. Recently, however, PERCIVAL[3] has shown that in the coleoptiles of an Indo-Abyssinian emmer wheat (*Triticum dicoccum* Schübl.) from two to six bundles may be present. He has construed the presence of these several bundles as evidence in favor of the coleoptile being a single leaf, and not double in origin.

Further evidence in favor of the single leaf theory, as set forth by PERCIVAL in *Triticum dicoccum*, is supplied by the writer's observations on some 15,000 seedlings of *Zea mays* L. The greater number of plants examined represented different strains of the varieties Golden Glow, Ninety-Day Yellow Dent, and Golden Bantam. In addition Dwarf, Liguleless, and Chinese Suckering strains were observed. Certain strains of the Golden Glow variety showed more than two bundles in the coleoptile. Two to five bundles were observed to be present (figs. 1–4). These additional bundles destroy the "double" symmetry of the coleoptile.

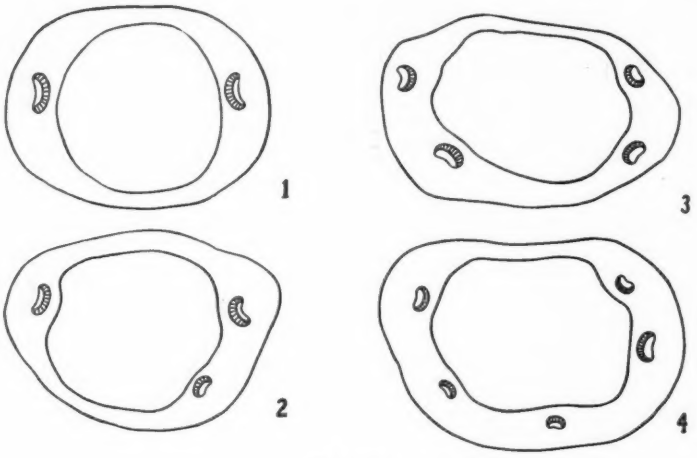
The position of the bud in the axil of the coleoptile should also be of some significance in determining whether it is a single structure,

[1] SARGANT, E., and ARBER, A., The comparative morphology of the embryo and seedling in the Gramineae. Ann. Botany **29**:161–222. 1915.

[2] WORSDELL, W. E., Morphology of the monocotyledonous embryo and that of the grass in particular. Ann. Botany **30**:509–524. 1916.

[3] PERCIVAL, J., The coleoptile bundles of Indo-Abyssinian emmer wheat (*Triticum dicoccum* Schübl). Ann. Botany **41**:101–105. 1927.

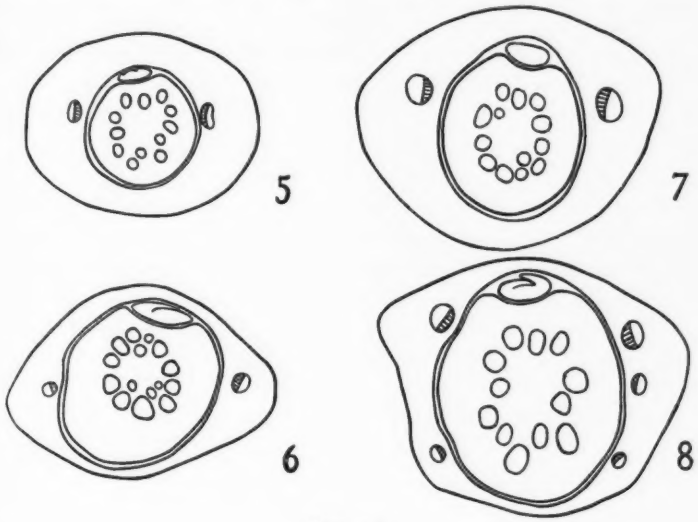
or represents the congenital union of two structures. If the bud is regularly alternate distichous with the buds that follow on the axis, it must bear the same relation to the coleoptile that later buds bear to the later leaves. If the coleoptile were actually a "double" structure, two buds might be expected in its axil instead of one, each bud in front of one of the two characteristic bundles. In no case was more than one bud found. In the cases of *Avena*, *Hordeum*, and *Triticum*, in all of which buds are usually found in the axil of the



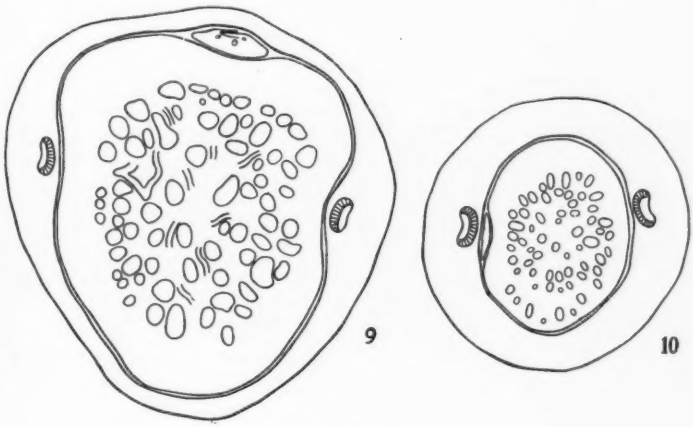
FIGS. 1–4

coleoptile, the bud occurs between the two bundles, on the same side of the axis as the scutellum and directly above its insertion (figs. 5–8). It has the same relative position with reference to the scutellum in *Triticum*, irrespective of the number of vascular bundles (figs. 7, 8). Buds are not commonly found in the axil of the coleoptile of maize. Of the large number of seedling plants examined, only three such buds were found, and these were in the Golden Glow variety. In no case was a bud found in the axil of a coleoptile that had more than the usual two bundles. In two of the three cases the buds occupied the same relative position as those in *Avena*, *Hordeum*, and *Triticum* (fig. 9). In the third case it occupied a position almost directly in front of one of the bundles of the coleoptile.

The fact that in one instance of maize a bud appeared in the axil of the coleoptile, almost directly in front of one of the bundles, does not seem significant. It will be noted that the buds observed in



FIGS. 5–8



FIGS. 9–10

the different grasses varied in position, being somewhat closer to one coleoptile bundle than to the other (figs. 5–8). A single case, such as shown in fig. 10, might be considered an extreme example of this variation. If the coleoptile is considered a single leaf, the bud in its axil bears exactly the same relation to it that later buds bear to later foliage leaves, except that the coleoptile has no midrib. The coleoptile is then alternate distichous with the later leaves.

There seems quite as much or more evidence to consider the coleoptile a single leaf, than the result of fusion of two stipules or other structures. If one considers the coleoptile the first green leaf of the plant,[4] it is not surprising that it differs from the later leaves. Such is also the case in a great many dicotyledons. In fact it is very often true that the first two or three leaves above the cotyledons differ markedly from the later leaves of the plant.

DUKE UNIVERSITY
DURHAM, N.C.

[*Accepted for publication February 7, 1928*]

[4] Under normal physiological conditions the cells of the coleoptile contain some chlorophyll, although such cells are usually confined to the vicinity of the vascular bundles.

BRIEFER ARTICLES

A STAIN COMBINATION FOR PHLOEM TISSUES OF WOODY PLANTS

(WITH ONE FIGURE)

Incident to research upon the phloem of our native woody plants, a Bismarck brown-Haidenhain's iron-haematoxylin combination has been found to give results more satisfactory than those obtained by the usual anilin safranin-haematoxylin schedule, and is therefore to be recommended. Fig. 1 is a transverse section of *Fraxinus pennsylvanica* Marsh, stained as indicated and photographed with a green ray filter at 250 diameters. The clearness of detail at this magnification, the stratification of the walls of the bast fibers, and the sharp demarcation between the sieve tubes and companion cells, ray cells, and phloem parenchyma cells should be noted. The dyes act as a triple stain, and have been used with equal success on the phloem of both hard and soft woods.

Staining schedule

1. Place sections in a 2 per cent aqueous solution of ferric ammonium sulphate for 20 minutes. If materials have been killed and fixed this time may be reduced by one-half.

2. Drain mordant and wash sections with five changes of distilled water to insure complete removal of excess mordant.

3. Flood sections with distilled water and add two or three drops of a 1 per cent aqueous solution of Haidenhain's iron-haematoxylin. Watch progress of stain under a microscope and stop at desired point by another change of water.

4. Drain off water and immerse section in a 1 per cent aqueous solution of Bismarck brown for three or four hours, depending on the thickness of the sections.

5. Drain off excess stain.

6. Dehydrate sections with the following changes of alcohol: 10, 20, 30, 50, 70, 95, and 100 per cent. Flood sections with two changes of 100 per cent alcohol to remove all traces of water.

7. Clear in xylol and mount in balsam.

The stone cells of hard woods stain a cherry red and the bast fibers a brilliant orange, the ray cells and other parenchymatous tissue a chestnut brown, and the middle lamella a dark blue. The bast fibers, parenchymatous tissues, and middle lamellas of conifers stain as indicated,

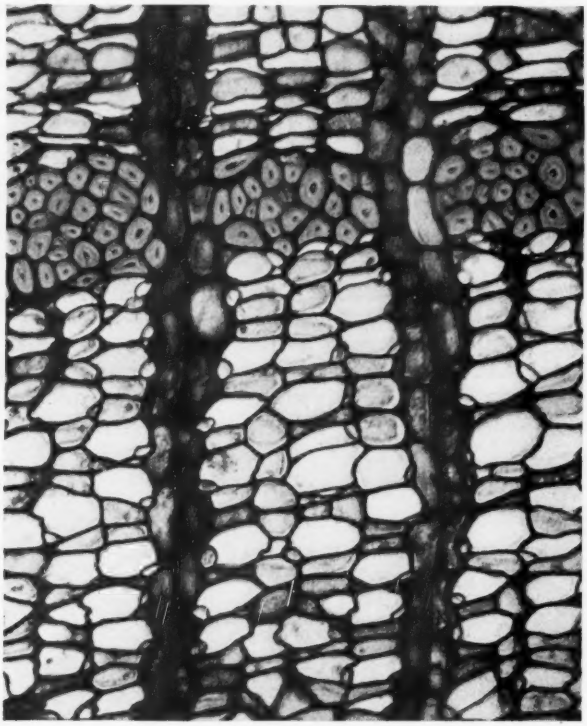


FIG. 1

while the stone cells turn a vivid burnt orange. Acknowledgments are due Dr. H. P. BROWN for suggestions in preparing the manuscript.— E. S. HARRAR, *New York State College of Forestry, Syracuse, N.Y.*

[*Accepted for publication January 7, 1928*]

ORIGIN OF ENDODERMIS IN FERNS

In his recent contribution on the origin and development of tissues in the rhizome of *Pteris aquilina*, CHANG (3) draws certain fundamental conclusions on the cell lineage of the various morphological elements. He shows that the pericycle and endodermis are respectively the inner and outer products of the tangential division of a single layer of meristematic cells, and are accordingly sister tissues. This alone could hardly be considered sufficient evidence that the endodermis arises from the plerome. STRASBURGER (7), who also recognized the common origin of endodermis and pericycle, referred both of these layers to the periblem. CHANG, however, further determined that the common mother cell layer of endodermis and pericycle on the one hand, and the protophloem on the other, are derived from the periclinal division of a single layer of cells; hence he is certainly justified in his remark that "no room is left for doubt of the stelar origin of both the endodermis and the pericycle."

As CHANG recognized, however, this statement is in direct contradiction to the generalizations of the current textbooks. CAMPBELL (2), in discussing the rhizome of the Polypodiaceae as a group, repeats STRASBURGER'S conclusion that both pericycle and endodermis are cortical in origin. JEFFREY (5), in describing the rhizome of *Pteris*, ascribes the endodermis to the cortex, and in such textbooks as those of STRASBURGER, and of COULTER, BARNES, and COWLES, the endodermis of the stem of vascular plants in general is treated under the heading of cortex, probably following the conclusions of VAN TIEGHEM and DULIOT. In consideration of these facts, it seems that CHANG would have been at pains to cite any work which lends support to his conclusion of the stelar origin of the endodermis. He does record the finding of SCHOUTE (6), confirmed by BARRATT (1), that in the stem of *Hippuris* the endodermis and several layers of the cortex are derived from the plerome; but, strangely enough, he neglects to mention the important monograph of CONARD (4) on the development of *Dicksonia* (*Dennstaedtia*) *punctilobula*. This writer demonstrated (in 1908) that the segments of the initial cell of the rhizome of this fern divide by periclinal walls into three layers, the outer one of which gives rise to the epidermis and cortex, the inner to the pith, and the middle to the plerome of the amphiphloic siphonostele. This latter divides by three periclinal walls to form four cells, of which the outer and the inner give rise respectively to the outer and inner endodermis and pericycle. The endodermis and pericycle, as revealed by actual mitoses as well as by the alignment of the mature cells, are sister

tissues which originate in the plerome. Thus we have independent evidence derived from two distinct genera of the Polypodiaceae with entirely different stelar types, the one siphonostelic and the other polystelic, of the stelar origin of the endodermis in the rhizome.

In the root of *Dicksonia*, on the other hand, the endodermis is cortical. The second pericline in each segment derived from the tetrahedral initial separates an inner cell which originates the plerome from an outer which gives rise to the endodermis and most of the cortex. The first periclinal division of this outer cell occurs near its inner wall, and cuts off the endodermis from the remainder of the cortex. The cortical affinity of the endodermis of the root has been demonstrated for a long list of ferns (including *Pteris aquilina*) which CONARD cites. In view of the direct continuity of the endodermis from the root where it is cortical, to the stem where it is stelar, it is evident that the sequence of cell walls in the apical meristem is of little importance in determining the subsequent morphological and physiological specialization of tissues.—ALEXANDER F. SKUTCH, *Johns Hopkins University, Baltimore, Md.*

[*Accepted for publication January 27, 1928*]

LITERATURE CITED

1. BARRATT, KATE, The origin of the endodermis in the stem of *Hippuris*. Ann. Botany **30**:91–99. 1916.
2. CAMPBELL, D. H., Mosses and ferns. 3d ed. New York. 1918.
3. CHANG, C. Y., Origin and development of tissues in rhizome of *Pteris aquilina*. BOT. GAZ. **83**:288–306. 1927.
4. CONARD, H. S., The structure and life-history of the hay-scented fern. Carnegie Inst. Washington. 1908.
5. JEFFREY, E. C., The anatomy of woody plants. Chicago. 1917.
6. SCHOUTE, J. C., Die Stelär-Theorie. Groningen. 1902.
7. STRASBURGER, E., Histologische Beiträge, III. Jena. 1891.

CURRENT LITERATURE

BOOK REVIEWS

Textbook of systematic botany

For many years the subject of systematic botany in American universities and colleges has suffered from the lack of a suitable text. This lack has been remedied in large part by a new volume by SWINGLE.[1]

The book is divided into two parts, the first part dealing with principles and methods, the second with a general discussion of the Spermatophyta and a special treatment of sixty representative families. The discussion of principles and methods is notable for its breadth of treatment and for its balanced perspective. It includes many phases of botany that at a former stage in the history of taxonomy were only too often neglected. Chapters upon evolution, principles of taxonomy, difficulties in classification, development of systems of classification, phylogeny of spermatophytes, and nomenclature serve to present the more theoretical aspects of taxonomy. These are followed by chapters of a somewhat more practical nature, dealing with the preparation of herbaria, also with the terminology and literature of systematic botany.

The volume is the outgrowth of some fifteen years' teaching by its author in a course given at the Montana State College. He appears throughout its pages to have kept in mind his intention to prepare primarily a textbook, and not a manual or some other more or less supplementary book. The general plan of presentation is excellent. The discussions are characterized by a commendable degree of fairness and conservatism. On the whole, it is probable that the introduction of this book to university and college classes will mark one of the most significant forward steps which the subject of taxonomy has taken in recent years.

As often happens with first editions, however, various inconsistencies or inaccuracies are present to detract from the value of the work. Some of these are apparently typographical slips. Thus VON BAER (p. 11) later becomes VON BAYER (p. 49). DECANDOLLE (p. 108) is DECANDOLE elsewhere (p. 29). This is hardly the case, however, with the many trivial names of geographic origin. Thus, *Asclepias Mexicana* (p. 200), *Rheum Rhaponticum* (pp. 146, 147), *Ulmus Americana* (p. 144), etc., have the trivial name's initial letter capitalized, while *Heliotropium peruvianum* (p. 203), *Magnolia virginiana* (p. 152), *Aeschynomene virginica* (p. 169), etc., do not. In *Xanthium* (p. 214) the writer appears to dis-

[1] SWINGLE, D. B., A textbook of systematic botany. pp. xiii+254. McGraw-Hill Book Co. 1928.

regard or overlook the staminate (and multiple) inflorescence entirely, and so attributes but two flowers to the inflorescence. VON BAER, already mentioned, is associated with a statement of the "law of recapitulation" (pp. 11, 49) in words that might more properly be ascribed to ERNST HAECKEL. The buds of *Salix* (p. 130) are confused with those of *Populus*. The nomenclature for specific names does not always conform to the latest findings of special research. Thus (p. 213) *Taraxacum officinale* is retained despite LAMARCK'S earlier *T. vulgare*. BESSEY'S view that opposite cauline leaves preceded alternate ones is correlated with the "fact that in dicotyledonous plants the first leaves (cotyledons) of the embryo are formed two at a node while in the monocotyledonous plants there is but one at a node." In view of researches tending to confirm the existence of a vestigial second cotyledon in various monocotyledonous seeds (mentioned by SWINGLE himself later on p. 220), the treatment given this matter appears inadequate and even misleading. One might wish also for more amplification in the chapter on nomenclature. Thus, for example, a discussion of homonyms and synonyms (p. 68) includes a treatment of *Lactuca integrifolia* Bigel., and *L. sagittifolia* Ell. is there given as the valid name to be adopted. SWINGLE might profitably add for the student's information that his reasoning in this case follows the American Code but directly violates the International Code, although indeed the pertinent article (no. 50) in the latter will doubtless be changed at some future date.

It is to be hoped that a second edition may enlarge upon certain subjects which are too much restricted in the first. Among herbaria, only one (Kew) outside the United States is listed (p. 75). In the chapter on literature there is no mention of some of the standard texts in systematic botany, such as WARMING'S well known handbook. Under a heading, "The Classics" (p. 98), BENTHAM and HOOKER'S *Genera Plantarum* is listed, but one looks in vain for several other fully as epochal works, such, for example, as DECANDOLLE'S *Prodromus*. The subject of Descriptive Manuals (p. 102) is very inadequately handled, several works, such, for example, as TIDESTROM'S *Flora of Utah and Nevada* having been omitted entirely. Under the heading (p. 113) of "Indexes, Catalogues, etc.," PRITZEL'S *Thesaurus* is described, but his *Icones*, a much larger work, is omitted; nor are the monumental works of STEUDEL, PFEIFFER, and certain other writers listed. The gigantic card index issued periodically from the Gray Herbarium is omitted, as also several other invaluable card indexes and finding lists. Great library catalogues such as those of Kew and the Arnold Arboretum are likewise omitted, nor is there mention of such really extensive works as the BRADLEY *Bibliography* and the International Catalogue of Scientific Literature, to name only two of several. It would seem that an expansion of the volume in these respects could in no way impair its usefulness for the average class of beginners in the subject, but would serve to adapt it almost equally well to a large number of more advanced students who may have reached the point of entering upon research.—E. E. SHERFF.

Microscopy of oil shales and coals

Two very useful books on the microscopy of oil shales and coals, and the technique of preparing thin sections and other microscopic preparations from these objects have recently been published. Their contents are of special interest to paleobotanists.

ROBERT POTONIÉ'S volume on oil shales and related substances[2] summarizes our present knowledge of bituminous shales, of which the largest group is formed by the oil shales. He discusses the principal types of these shales, and accompanies his descriptions with ample microphotographic illustrations. The organisms which supplied the bituminous substances in the shales are discussed, and as far as possible determined. The author makes numerous original contributions and uses the literature very extensively. It is noted that many algal forms besides animal substances have produced oil in oil shales, and in some instances these algae can still be identified.

The microscopic examination of coal is discussed in detail by STACH.[3] He summarizes our present knowledge of the microscopic technique in coal examinations. The main chapter deals with the preparation of thin sections. The methods of LOMAX, THIESSEN, and JEFFREY are described in detail. There are also chapters on maceration and on microchemical investigation. The microscopic investigation with reflected light on the metallurgical microscope receives special consideration. There are chapters on microphotography of coal preparations, and on the microscopic determination of the various types of coal. The book is amply illustrated and contains a very complete bibliography.

Of special interest is a discussion of the three principal substances which constitute coal. These are: (1) the lustrous portion commonly called vitrit, which is primarily composed of wood; (2) the dull coal, durit, which contains primarily the exines and membranes of spores; and (3) the fiber coal, fusit, which originates from wood, like vitrit. Wherein the exact difference between vitrit and fusit lies has not yet been fully established.

The illustrations of STACH'S book demonstrate how numerous, and in many instances how well preserved are the vegetable organs of which coal is composed. His treatise is an extremely interesting contribution to the study of botanical micropaleontology, a very important branch of a science which is now being greatly developed, namely, micropaleontology in general. Its zoological as well as its botanical side promises to be not only of great scientific value, but also to possess numerous practical applications in the study of our mineral fuels and of their origin.—A. C. NOÉ.

---

[2] POTONIÉ, R., Allgemeine Petrographie der Ölschiefer und ihrer Verwandten. pp. 173. *figs.* 27. Berlin: Gebrüder Borntraeger. 1928.

[3] STACH, E., Kohlenpetrographisches Praktikum. pp. 196. *figs.* 64. Berlin: Gebrüder Borntraeger. 1928.

Filterable viruses

A very useful book for those interested in plant diseases, especially the so-called virus diseases, has been prepared under the editorship of RIVERS.[4] Although only one chapter applies immediately to plants, any one interested in the virus disease problem as found in plants will find it profitable to read the other chapters.

The book contains ten chapters. The first, by RIVERS, takes up the general aspects of filterable viruses. Filters and filtrations are discussed in the second chapter by MUDD. The third chapter, by CARREL, is devoted to a discussion of the use of tissue cultures in the study of viruses. COWDRY, in the fourth chapter, presents the intracellular pathology in virus diseases. Following these general topics, specific virus problems are discussed as follows: virus diseases of man as exemplified by foot and mouth disease and vesicular stomatitis, by OLITSKY; virus diseases as exemplified by contagious epithelioma (fowl-pox) of chickens and pigeons, by GOODPASTURE; virus diseases of insects (sacbrood of honey bees and the polyhedral disease), by GLASER; virus diseases of plants, by KUNKEL; and virus diseases of bacteria (bacteriophagy), by BRONFENBRENNER. Some of the chapters are well illustrated, and all carry extensive bibliographies. This feature alone makes the book a valuable contribution.

The volume is a most timely and welcome contribution in an interesting and perplexing field of biology, by men preeminently fitted for the task.—G. K. K. LINK.

Physiology of bacteria

The first volume of a series by BUCHANAN and FULMER[5] on the physiology and biochemistry of bacteria has made its appearance. This volume will be of interest, not only to the bacteriologist, but also to the mycologist, for in spite of the title some attention is paid to those fungi which the bacteriologist classes as "molds and yeasts."

The book consists of five chapters, of which the first is a brief discussion of the scope of physiological bacteriology. The second chapter deals with the growth phases and growth rates of microorganisms in cultures. The remaining chapters are devoted to a discussion of the chemical composition, energetics, growth and movement of microorganisms, and the physico-chemical and physical characteristics of microorganisms and of their environment.

The authors have done a real service in very ably compiling and systematizing the extensive material relating to the physiology of microorganisms which has hitherto been widely scattered in numerous publications. The next volume is awaited with interest.—G. K. K. LINK.

---

[4] Edited by RIVERS, T., Filterable viruses. 8vo. ix+428. Baltimore: Williams and Wilkins Co. 1928.

[5] BUCHANAN, R. E., and FULMER, E. I., Physiology and biochemistry of bacteria. Vol. I. xi+516. *figs.* 78. Baltimore: Williams and Wilkins Co. 1928.

NOTES FOR STUDENTS

Mitogenetic rays.—One of the most remarkable theories of recent years regarding cell division is that of the "mitogenetic rays" of GURWITSCH.[6] These rays are claimed to be emitted from actively dividing root tip cells, and to induce mitosis in neighboring roots. They are said to pass through air and quartz but to be stopped by glass and by a quartz plate which has been coated with gelatine. They are thus considered to be of the nature of ultraviolet light, and to have a wave length of 1900–2000 Ångström units.

These conclusions are based upon the following type of experiment. The meristematic region of a horizontally growing onion root tip was placed a few millimeters from the same region of a root growing vertically downward. After at least twenty minutes the vertically placed tip was sectioned longitudinally, when many more mitoses were found on the side nearest the horizontal tip than on that farthest from it. These reports have not received much attention, probably because there seems to be no corroboration of them in our biological knowledge derived from other sources. It is highly important, however, that the truth about these rays should be established, and this appears to have been done in a recent paper. GUTTENBERG[7] first points out the inadequacy of GURWITSCH'S own data for a critical examination of the problem. The zones studied were not exactly indicated, nor were the actual counts given in any complete or thorough manner. Figures were given in percentage of increase or numerical differences. Moreover details were not published as to actual phases of mitosis and the extent to which they varied in excess over corresponding stages on the opposite side of the root.

GUTTENBERG'S own work appears to have been carefully conceived and elaborately carried out. *Pisum* as well as *Allium* was studied, isolated vertically growing roots were used as controls, and all mitotic phases were recorded separately. The first difficulty encountered was to determine accurately the beginning of prophase. Counts by different observers and by the same observer at different times convinced the author that this point could not be determined objectively; hence a great source of error arose in early prophase counts. Spireme, metaphase, anaphase, and telophase of course could be detected with certainty.

A point of note is made regarding median sections. Here many more cells (and so more nuclei) are seen than in tangential sections, and so more cases of division. Thus with higher totals greater deviations in number are to be expected. This would not give a greater percentage difference, however, and on this basis the results would be more accurate than when the totals are fewer.

---

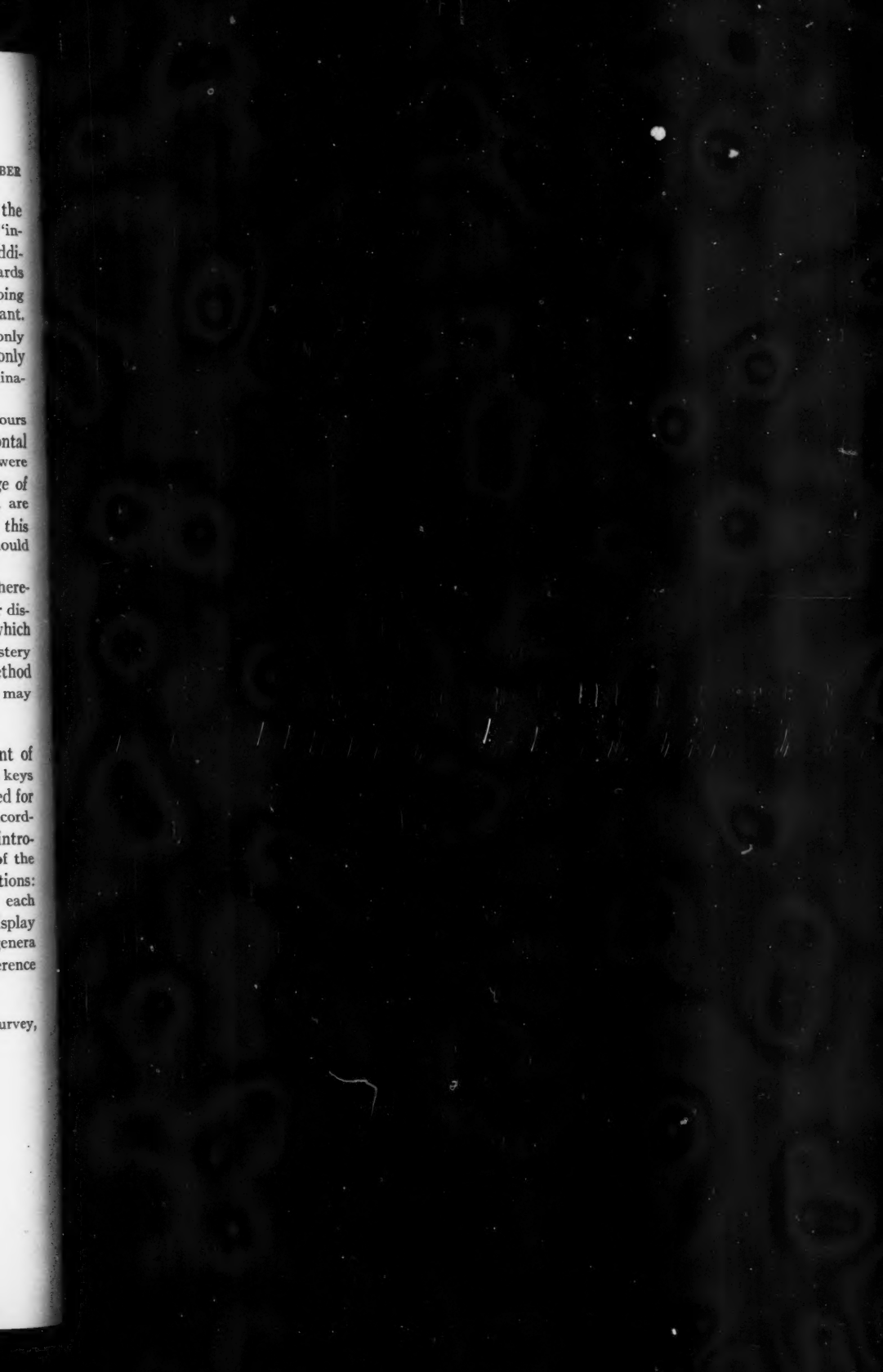
[6] GURWITSCH, A., Das Problem der Zellteilung physiologisch betrachtet. Berlin. Verlag Julius Springer. 1926.

[7] GUTTENBERG, HERMANN VON, Die Theorie der mitogenetischen Strahlen. Biologisches Zentralblatt 48:31–39. 1928.

GUTTENBERG has made many thousands of counts, and has recorded the results in tables with phases shown separately. Both actual counts on the "induced" and opposite sides and differences between them are shown, with additional tables for median sections. From these it is apparent that as regards numbers of cells in any given phase, and totals of all cells definitely undergoing division, the differences between one side and the other are not significant. Sometimes there is an excess on one side, sometimes on the other. The only exception is in early prophase, where a greater number were more commonly counted on the "induced" side. This is the stage where the errors in determination are very great.

Further work was done on roots exposed from one and a half to two hours with fixation at various times thereafter, and also with more than one horizontal root. The results were similar to those given. Moreover, growing root tips were found not to affect photographic plates especially prepared for the range of ultraviolet light. GURWITSCH's claim that spectral rays of 1930–2370A are effective in inducing mitosis was not investigated, but the author feels that this also is not proved because of the method of counting. Moreover, if this should be established, it would not prove that roots emit such rays.

GUTTENBERG appears to have made his case. "Mitogenetic rays" are therefore due to join company with phlogiston, abiogenesis, "n rays" and other discarded theories. It is a great misfortune that mitosis, the process upon which the persistence of protoplasmic types depends, should remain so great a mystery in its fundamental principles. However, trial and error is the traditional method of advance, and it is devoutly to be wished that some element of success may soon crown our efforts.—R. O. EARL.

Algae of Connecticut.—HYLANDER[8] has published a very full account of the algae of Connecticut. The species are not described, but the numerous keys enable the reader to place the genera. Every county of the state was visited for material. It is recorded that "out of the 166 towns in the state, 123 have recorded algae stations." This certainly indicates a very thorough survey. The introductory pages give a full account of the morphology and classification of the algae. The classification is then presented under the following usual sections: Myxophyceae, Chlorophyceae, Phaeophyceae, Rhodophyceae. Under each species all the localities are cited where material has been collected. The display of algal flora in the state is very large, as shown by the great number of genera and species. This publication will serve for a long time as a standard of reference for students of this group in Connecticut.—J. M. C.

[8] HYLANDER, C. J., The algae of Connecticut. Conn. Geol. & Nat. Hist. Survey, Bull. 42. pp. 245. 1928.